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Synthesis and antimicrobial activity of amide derivatives of polyether antibiotic—salinomycin

Adam Huczyński [a,*], Jan Janczak [b], Joanna Stefańska [c], Michał Antoszczak [a], Bogumil Brzezinski [a]

[a] Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland
[b] Institute of Low Temperature and Structure Research, Polish Academy of Sciences, PO Box 1410, 50-950 Wrocław, Poland
[c] Medical University of Warsaw, Department of Pharmaceutical Microbiology, Oczki 3, 02-007 Warsaw, Poland

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ABSTRACT

For the first time a direct and practical approach to the synthesis of eight amide derivatives of polyether antibiotic—salinomycin is described. The structure of allyl amide (**3a**) has been determined using X-ray diffraction. Salinomycin and its amide derivatives have been screened for their in vitro antimicrobial activity against the typical Gram-positive cocci, Gram-negative rods and yeast-like organisms, as well as against a series of clinical isolates of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *S. aureus*. Amides of salinomycin have been found to show a wide range of activities, from inactive at 256 μg/mL to active with MIC of 2 μg/mL, comparable with salinomycin. As a result, phenyl amide (**3b**) was found to be the most active salinomycin derivative against Gram-positive bacteria, MRSA and MSSA.

Salinomycin (**2**) (Scheme 1) is a natural carboxylic polyether antibiotic isolated from *Streptomyces albus*.[1] Salinomycin and its salts exist in a pseudo-cyclic structure due to the formation of hydrogen bonds between the carboxylic group on the one side of the molecule and two hydroxyl groups on the opposite side.[2] The polyether skeleton of the pseudo-cyclic structure is able to form complexes with metal cations and transport them across lipid membranes.[3]

Recently it has been shown that in addition to its antibiotic activity, **2** selectively kills breast cancer stem cells.[4] Further studies have also shown that salinomycin can act as a *P-gp* inhibitor to overcome apoptosis resistance in human cancer cells, including leukaemia stem cell-like cells.[5] Salinomycin also inhibits chemoresistant cancer cells by increasing apoptosis causing DNA damage and reducing p21 protein levels through increased proteasome activity. Moreover, **2** inhibits Wnt signalling and selectively induces apoptosis in chronic lymphocytic leukaemia cells.[5–10] Therefore, at present **2** is considered to be a potential anti-cancer drug for cancer chemoprevention and cancer therapy.[11]

Results of the aforementioned studies have prompted organic chemists to search for a new group of **2** derivatives, which will be more antimicrobial and anticancer active and more effective in the coordination of metal cations and less toxic especially to humans. Up to now only a few modifications of **2** have been developed to obtain its salts with monovalent[12] and divalent[13] metal cations and its *p*-iodophenacyl derivative.[14]

In an earlier paper we have described the synthesis, structural studies and antibacterial activity of unexpectedly stable benzotriazole ester of **2.**[15] The antimicrobial tests have shown that this salinomycin derivatives is active against Gram-positive bacteria and clinical isolates of methicillin-resistant *Staphylococcus aureus* (MIC = 1–2 μg/ml).[15]

In this contribution, we present an efficient method for the synthesis of salinomycin amides, which is an interesting novelty as up to now no amide derivatives of **2** have been described. The structures of new amides of **2** were evaluated using X-ray and spectroscopic methods. The antimicrobial activity of **2** and its derivatives especially against a series of clinical isolates of *Staphylococcus*: methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) were be also studied and discussed here. Methicillin-resistant *S. aureus* (MRSA) is probably the best-known example of a resistant bacterium and has been the focus of intense scientific interest around the world.

Our previous investigation of semi-synthetic derivatives of polyether ionophores has shown that the chemical modification of this class of antibiotics can change the ability and selectivity of binding to metal cations, modify the mechanism of the cation transport from electroneutral to electrogenic and lead to new antibacterial and antifungal active compounds.[16–21] The chemical modification of salinomycin can lead to compounds which will

* Corresponding author. Fax: +44 1223 336 033.
E-mail address: adhucz@amu.edu.pl (A. Huczyński).

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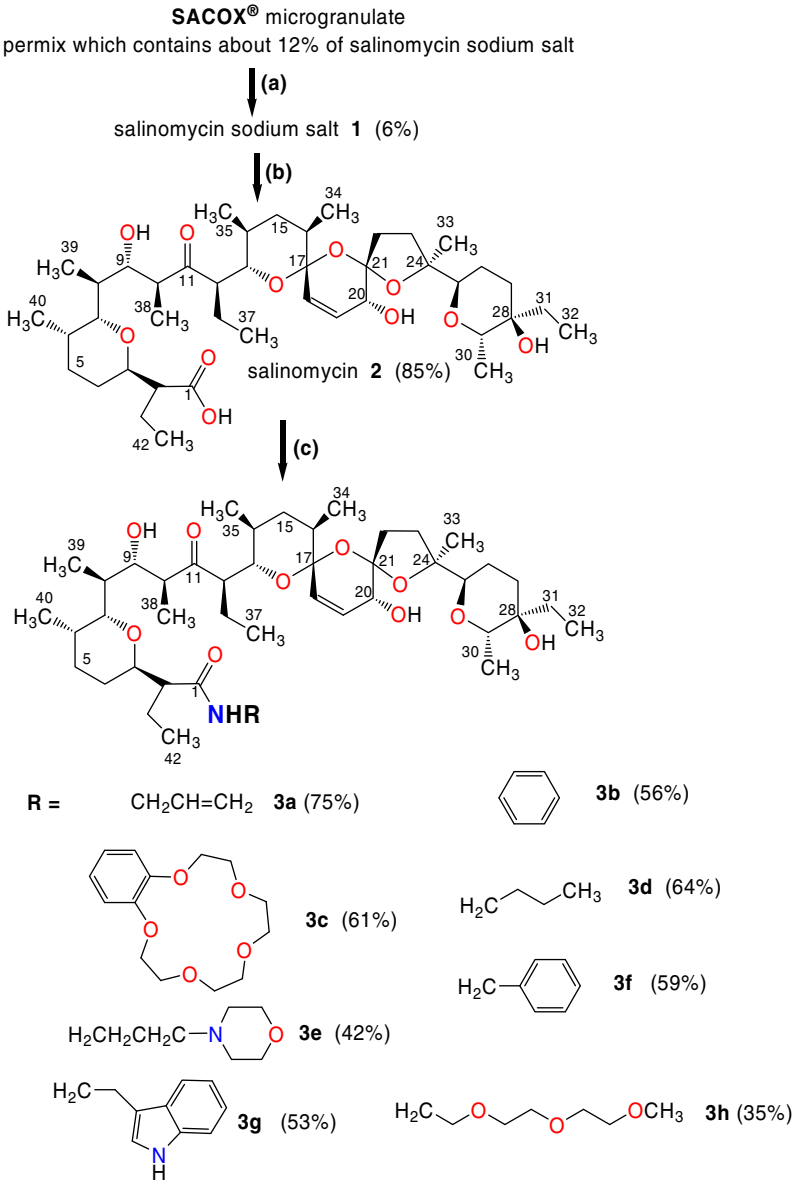

Scheme 1. Reagents and conditions: (a) suspension in CH_2Cl_2, purification by Dry Column Vacuum Chromatography on silica gel; (b) extraction CH_2Cl_2/H_2SO_4 (pH 1.5); (c), DCC, HOBt, appropriate amine, CH_2Cl_2/THF (3/1), 0 °C – 1 h, then rt to 24 h. Time for completion of the reaction at rt as indicated by TLC. Yield of isolated and purified products. For the experimental procedure, see Ref. 18.

be more effective in binding cations and be more antibacterial and anticancer active and finally can be also less toxic for humans.

Salinomycin sodium salt (**1**) was isolated from premix—SACOX® (commercially veterinary feed additive) which contains about 12% of **1** and purified using Dry Column Vacuum Chromatography.[22] The structure and homogeneity of isolated **1** was confirmed using spectroscopic methods (Figs. S1–S5, Supplementary data).[23] Salinomycin carboxylic acid (**2**) was obtained from salinomycin sodium salt by the extraction with H_2SO_4 (pH 1.5) in CH_2Cl_2.[24]

The synthesis of amides is one of the most fundamental methods in organic chemistry used to obtain natural and synthetically useful compounds.[25,26] A lot of procedures of amidation require rather rigorous conditions for example, synthesis from acyl chlorides. However, salinomycin is very sensitive to acidic conditions and heat. For this reason we chose mild reaction conditions for the amidation. A number of methods for the preparation of primary amides starting from carboxylic acid have been reported.[26] Due to the accessibility and versatility of DCC (*N,N'*-dicyclohexylcarbodiimide) and HOBt (1-hydroxybenzotriazole) we used these compounds in our synthetic procedure. The new salinomycin amides (**3a**–**3i**) were prepared according to a protocol developed by our group.[27]

The respective synthesis pathways are summarized in Scheme 1. All amides can be easily isolated in pure form after purification by Dry Column Vacuum Chromatography. This method was efficient and gave the amides in moderate to good yields (35–75%) of pure product (Scheme 1). The purity and structures of the amides were determined on the basis of elemental analysis, FT-IR and NMR methods (exemplary spectra are included in Supplementary data, Figs. S5–S11).[27] For the synthesis of salinomycin derivatives, the commercially available amines were used giving their amides with different substituents such as: unsaturated alkyl chain (allyl amide, **3a**), aromatic ring (phenylamide, **3b**), crown ether (amide of benzo-15-crown-5, **3c**), alkyl chain (butylamide, **3d**), alkyl chain containing heterocyclic ring (3-morpholine-propylamide, **3e**), alkyl-aromatic group (benzylamide, **3f**), biogenic amine (tryptamine

amide, **3g**) and alkyl chain containing etheric oxygen atoms (3,6,9-trioxadecylamide, **3h**).

It is worth noting that the use of only DCC as a coupling agent in the syntheses of salinomycin amides yielded no desired product. A similar result was achieved by us previously in attempts to obtain amides and esters of monensin A.[17–19] In one case, *i.e.* in the reaction between salinomycin acid and 1-naphthylamine at room temperature with the addition of DCC and HOBt, the expected amide product was not observed. Detailed analysis of the collected series of fractions from flash chromatography allowed us to discover an unexpected by-product (30% yield). This product is an intermediate HOBt ester and its crystal structure has been describe by us previously[15]

In Figure 1 the FT-IR spectrum of salinomycin sodium salt (**1**) (solid line) is compared with that of salinomycin acid (**2**) (dashed line) and that of salinomycin allyl amide (**3a**) (dashed-dotted line) and clear differences between these structures are revealed. In the FT-IR spectra of **1** and **2** the maximum of the broad band assigned to ν(O–H) stretching vibration of three hydroxyl groups present in the salinomycin molecule is observed at 3373 cm^{-1} and 3484 cm^{-1}, respectively, indicating different hydrogen bonds existing within their structures. In the spectrum of **1**, the bands assigned to the $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ vibrations are found at 1565 and 1404 cm^{-1}, respectively. In the spectrum of **2** these bands are not observed and a new broad band arises with a maximum at about 1713 cm^{-1} due to overlapping of the ν(C=O) vibrations of both ketone and carboxylic groups. In the spectrum of **3a** two intense bands at 1665 and 1528 cm^{-1} are assigned to the amide I band and amide II band, respectively, indicating the formation of the amide bond.

In the ^{13}C NMR (all in CD_2Cl_2) spectra of salinomycin amides, the most characteristic signal of C(1) atom of the amide group was observed in a narrow range 175.0–175.8 ppm, while the signal of C(1) atom of the carboxyl group of salinomycin acid (**2**) was at 177.8 ppm and the signal of the carboxylic group of salinomycin sodium salt (**1**) was at 185.0 ppm. In the ^1H NMR spectra of salinomycin amides, the characteristic signals of the N–H proton were observed between 6.34–8.52 ppm.

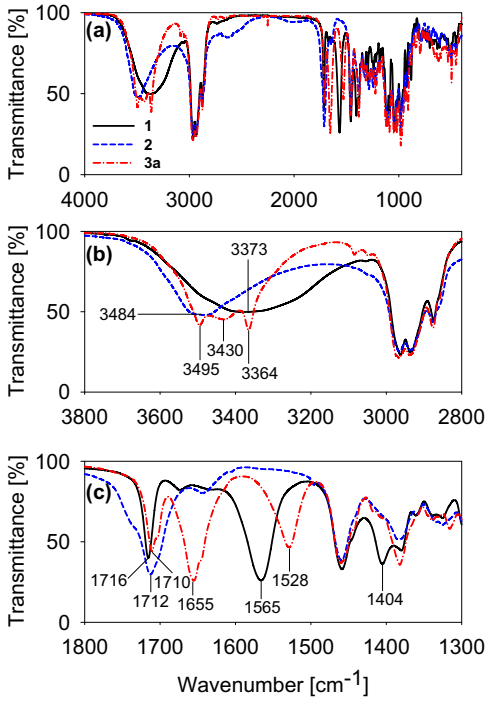

Figure 1. The FT-IR spectra of crystal of **1** (—), **2** (- - -) and **3a** (-·-) recorded in KBr pellet: (a) 4000–400 cm^{-1}; (b) 3800–2800 cm^{-1}; (c) 1800–1300 cm^{-1}.

Structural data of salinomycin derivatives such as amides can be very useful when describing their anticancer and antimicrobial properties as well as for structural–activity relationship analysis (SAR) and related investigations. We have therefore characterized **3a** by single crystal X-ray diffraction methods. Single crystals of **3a** were grown by crystallisation in acetonitrile solution as acetonitrile solvate and an X-ray crystallographic analysis confirmed the structural assignment (Fig. 2). The absolute configuration of **3a** is (*2R,3R,6S,7R,8S,9S,10S,12R,13S,14S,16R,17R,20R,21S,24S,25R,28R,29S*) and it was analogously determined for salinomycin.[1,2] The pseudo-cyclic conformation of **3a** is stabilised by two intramolecular N1–H···O3 and O3–H···O8 hydrogen bonds (Table 2). Three other intramolecular O–H···O hydrogen bonds (O7–H···O8, O7–H···O9 and O10–H···O9) result from the interaction between the hydroxyl groups (O7–H and O10–H) with the oxygen atoms of negative polarity. The six-membered rings of **3a** exhibit chair conformations, in which the bond lengths and angles do not differ significantly from the normal values. The solvent acetonitrile molecules are located in the crystal in the voids between the pseudo-cyclic molecules of **3a** (Fig. S12, Supplementary data). The intermolecular O10–H···O1^i hydrogen bonds between the terminal hydroxyl group (O10–H) of one molecule and the carbonyl atom of the amide group of the neighbouring molecule, together with the van der Waals forces, stabilise the arrangement of **3a** molecules in the crystal (Table 1).

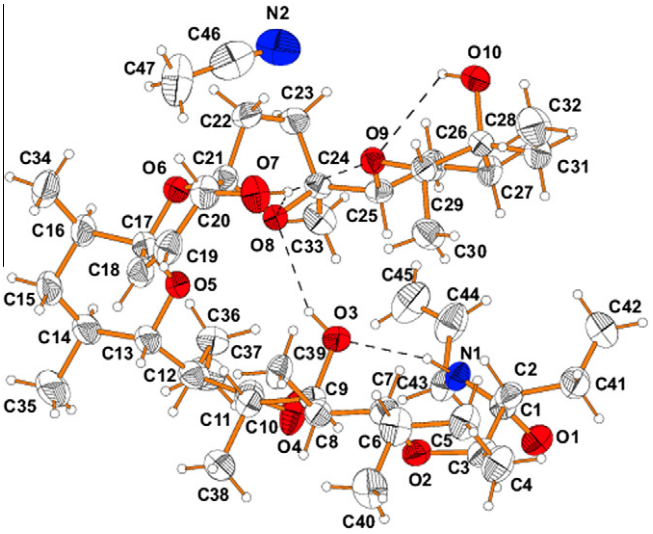

Figure 2. View of the molecular structure of salinomycin allyl amide acetonitrile solvate (**3a**·CH_3CN) with the atoms labelling. Dashed lines represent the hydrogen bonds.

Table 1
Crystal data of **3a**·CH_3CN

Salinomycin allyl amide (**3a**·CH_3CN)	
$C_{45}H_{75}NO_{10}$ CH_3CN	Dx = 1.157 Mg m^{-3}
M_r = 831.11	Dm = 1.15 Mg m^{-3}, D_m measured by Floatation
Monoclinic, $P2_1$	Mo $K\alpha$ radiation, λ = 0.71073 Å
a = 10.296 (2) Å	θ = 2.7–29.3°
b = 21.335 (4) Å	μ = 0.08 mm^{-1}
c = 11.076 (2) Å	T = 295 K
β = 101.26 (1)°	Parallelepiped, Colourless, 0.32 × 0.29 × 0.20 mm
V = 2386.2 (8) $Å^3$	$R[F^2 > 2\sigma(F^2)]$ = 0.0507, $wR(F^2)$ = 0.1283
Z = 2	S = 1.042
$F(000)$ = 908	Flack parameter: 0.2(2)

Table 2
Hydrogen-bond geometry (Å, °)

Crystal	D–H···A	D–H	H···A	D···A	D–H···A
Salinomycin allyl amide (**3a**)	O3–H3···O8	0.89(3)	2.22(3)	3.016(2)	148(3)
	O7–H7···O9	0.84(4)	2.27(5)	3.093(3)	166(4)
	O7–H7···O8	0.84(4)	2.26(5)	2.695(3)	112(4)
	O10–H10···O1^{i}	0.91(4)	2.11(4)	2.862(3)	139(3)
	O10–H10···O9	0.91(4)	2.49(4)	2.908(2)	108(3)
	N1–H1···O3	1.01(3)	2.15(3)	3.080(3)	154(3)

Symmetry code: (i) x−1, y, z.

The FT-IR spectra of salinomycin allyl amide (**3a**) in dichloromethane and in the solid are compared in Fig. S13 (Supplementary data). A comparison of these spectra shows only small changes. In the region between 1800 and 1400 cm^{-1} (Fig. S13c) the bands assigned to amide I and amid II are shifted towards higher and smaller wavenumbers, respectively and in the region over 3050 cm^{-1} (Fig. S13b) a new band at 3586 cm^{-1} arises. These spectral changes together with the NOESY spectrum of **3a** (Fig. S11) demonstrate that the intermolecular O(10)–H···O(1)=C(1) hydrogen bond becomes broken, whereby the intramolecular hydrogen bonds influencing the formation of the pseudo-cyclic structure are conserved. The fact that the pseudo-cyclic structures of the salinomycin amides are stable in the solid state and in solution can have strong biological implications, because this pseudo-cyclic structure of salinomycin is the reason for its high ability to form stable complexes with metal cation which is strictly connected with the mechanism of action and its biological activity. The stability of conformation of the salinomycin amide molecules in the solution was also confirmed by the multiplicity of the ^{1}H signals. Only for rigid molecules can all the spin-spin coupling (*e.g. tddd*, *ddd*, *qd*, *ddt*) be seen in the ^{1}H NMR spectrum as for instance in the spectrum of **3a** (Fig. S14, Supplementary data).

The antimicrobial activities of salinomycin (**2**) and its amides (**3a**–**3h**) were studied in vitro against the typical bacterial strains and yeast-like organisms[23] as well as against a series of clinical isolates of *Staphylococcus*: methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA). The antimicrobial properties of salinomycin and its derivatives against typical bacteria strain are expressed by the minimum inhibitory concentration (MIC) as well as by the growth inhibition zone (Giz) (Tables 3 and 4). Antibacterial activity evaluation largely depended on various substitutions in the amide moiety of salinomycin derivatives. Attempts were made to correlate antibacterial activity of these compounds with changing substitution at amide moiety.

Hospital strains of methicillin-resistant *Staphylococcus* were isolated from patients of the Warsaw Medical University Hospital. *S. aureus* is a species of bacterium commonly found on the skin

Table 3
Antibacterial activity of salinomycin (**2**), its amides (**3a–3h**). Data are given as diameter of Giz (mm) and MIC (μg/ml)[28,29]

	(**2**)		(**3b**)		(**3c**)		(**3e**)		Ciprofloxacin	
	Giz	MIC	Giz	MIC	Giz	MIC	Giz	MIC	Giz	MIC
S. aureus NCTC 4163	33	2	19	2	15	32	12	128	26	0.5
S. aureus ATCC 25923	31	2	18	2	13	32	11	128	26	0.5
S. aureus ATCC 6538	30	2	20	2	16	32	13	128	28	0.5
S. aureus ATCC 29213	29	2	21	2	13	32	11	128	22	0.25
S. epidermidis ATCC 12228	28	2	22	2	13	32	11	128	30	0.5
S. epidermidis ATCC 35984	30	2	20	4	12	64	11	256	32	0.25

Amides **3a**, **3d**, **3f–3h** were practically inactive towards all micro-organisms tested (Giz 10–12 mm and MIC ⩾256 μg/ml). Ciprofloxacin (control compound) is a synthetic antibiotic of the fluoroquinolone drug class. Salinomycin derivatives were inactive against the tested strains of *Candida* and Gram-negative rods[28].

Table 4
Antibacterial activity of salinomycin (**2**), its amides (**3b, 3c, 3e**) against hospital strains MRSA and MSSA (MIC (μg/ml))[28,29,32]

	(**2**)	(**3b**)	(**3c**)	(**3e**)	Monensin
Hospital strains of methicillin-resistant Staphylococcus aureus (MRSA)					
S. aureus 393/10	2	4	256	64	4
S. aureus 394/10	2	4	256	64	4
S. aureus 399/10	1	4	256	64	2
S. aureus 400/10	1	4	256	64	2
S. aureus 401/10	1	4	256	64	2
S. aureus 450/11	2	4	256	64	2
S. aureus 451/11	2	4	128	32	4
S. aureus 452/11	1	4	128	32	2
S. aureus 481/11	1	4	256	64	2
S. aureus 482/11	2	4	256	64	2
Hospital strains of methicillin-sensitive Staphylococcus aureus (MSSA)					
S. aureus 440/11	1	2	128	32	2
S. aureus 441/11	2	2	256	64	4
S. aureus 442/11	2	2	128	32	2
S. aureus 443/11	2	2	128	32	4
S. aureus 444/11	2	2	256	32	4
S. aureus 445/11	1	2	256	32	2
S. aureus 446/11	1	2	256	64	2
S. aureus 447/11	2	2	256	32	2
S. aureus 448/11	2	2	256	32	2
S. aureus 449/11	1	2	256	32	2

Monensin (control compound) is a natural antibiotic of the polyether ionophore drug class.

and/or in the noses of healthy people. Although it is usually harmless at these sites, it may occasionally get into the body and cause infections. These infections may be serious such as infection of the bloodstream, bones or joints. MRSA stands for methicillin-resistant which is a type of *S. aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class.[29–31] The data (MICs) concerning the antimicrobial activity against MRSA and MSSA of the compounds are summarized in Tables 3 and 4.

Among the compounds tested, only salinomycin A (**2**) as well as three amide derivatives (**3b**, **3c** and **3e**) showed activity against Gram-positive bacteria. Compounds (**3a**, **3d, 3f-3h**) were practically inactive towards all micro-organisms tested (MIC $\geqslant$256 μg/ml). Amides **3b** and **3c**, containing aromatic substituent benzyl and benzo-15-crown-5 group respectively, show a considerably better activity against Gram-positive bacteria than the corresponding aliphatic amides. Amide **3a** with benzyl substituent shows a considerably better activity against hospital strains of MRSA and MSSA than the corresponding active amides **3c** and **3e** indicating that the benzyl substituent in the amide moiety has a strong influence on the antibacterial activity of salinomycin amides. Among the compounds tested, only amide **3c** showed relatively strong activity against the series of clinical isolates of *Staphylococcus*: MRSA and MSSA (MIC = 2–4 μg/ml) and its activity is comparable with the activity of parent antibiotic. Salinomycin and its amide derivatives are inactive against strains of *Candida* and Gram-negative bacteria.

To summarize, for the first time a simple and efficient one-pot protocol for the synthesis of amides of anticancer antibiotic salinomycin has been described. The simplicity of this method, high yields, easy work-up and purification of the products by flash chromatography and crystallization are key advantages. These results are important for the development of molecules with dual potential anticancer and antibacterial activity.

We have provided evidence that the some of new derivatives of salinomycin show antibacterial activity against human pathogenic bacteria including drug resistant strains of *Staphylococcus aureus*. From among the compounds tested, phenyl amide **3b** was found to be the most active derivative against clinical isolates of *Staphylococcus*, being as effective against these organisms as the standard ionophore antibiotic monensin A.

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Supplementary data

Supplementary data (exemplary NMR, FT-IR spectra and figures) associated with this article can be found, in the online version. Details on data collection and refinement, fractional atomic coordinates, anisotropic displacement parameters and full list of bond lengths and angles in CIF format have been deposited at the Cambridge Crystallographic Data Centre, No. CCDC 865743 for the **3a**. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336 033; email: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.081.

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23. Salinomycin sodium salt (**1**) was isolated from Sacox® 120 micro Granulate an anticoccidial feed additive distributed by Huvepharma Polska.100 g of permix was dissolved in dichloromethane. The solvent was evaporated under reduced pressure and the crude product obtained was purified by Dry Column Vacuum Chromatography[16] (gradient solvent mixture hexane/dichloromethane) giving 6 g pure SAL-Na. ^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 6.03 (d, J = 9.28 Hz, 1H), 5.75 (d, J = 10.71 Hz, 1H), 5.20 (s, 1H), 5.04 (s, 1H), 4.05 (d, J = 10.28 Hz, 1 H), 3.99 (t, J = 6.40 Hz, 1 H), 3.68 (dd, J = 10.55, 5.27 Hz, 1 H), 3.62 (d, J = 9.88 Hz, 1 H), 3.54 (d, J = 10.01 Hz, 1 H), 3.41 (d, J = 12.16 Hz, 1 H), 2.66 (t, J = 11.84 Hz, 1H), 2.18-2.07 (m, 2H), 1.91 (t, J = 9.96 Hz, 2H), 1.88-1.06 (m, 47 H, signals are strongly overlapped), 0.85 (t, J = 6.72, Hz, 3 H), 0.82 (dd, J = 14.33, 7.35 Hz, 3H,) 0.73 (t, J = 8.18 Hz, 3H), 0.64 (t, J = 7.21 Hz, 3H), 13C NMR (75 MHz, DMSO-d6)δ ppm: 215.2, 181.4, 130.6, 122.0, 106.0, 98.4, 87.4, 75.7, 75.1,73.8, 70.4, 69.4, 66.7, 65.2, 54.8, 49.7, 48.4, 40.0, 38,1, 35.9, 35.7, 32.1, 31.9, 31.6, 28.7, 27.4, 26.8, 26.3, 23.1, 19.7, 19.3,17.4, 15.8, 15,7,14.5, 12.9, 12.5, 12.4, 10.9, 6.7, 6.2; The NMR data is agreement with previously published assignments2; FT-IR (KBr, cm–1), 3370 broad ν(O–H) band, 1716 ν(C(11)=O), 1566 νas(COO–), 1406 νs(COO–); Elemental analysis calc. for $C_{42}H_{69}O_{11}Na$: C, 65.26; H, 9.00; Found: 65.03; H, 9.29.
24. Salinomycin acid (**2**). Salinomycin sodium salt (**1**) was dissolved in dichloromethane and stirred vigorously with a layer of aqueous sulphuric acid (0.015 mol dm^{-3}) 3 times. The organic layer containing **2** was washed with distilled water, and dichloromethane evaporated under reduced pressure to dryness to produce the acid. The spectroscopic data of **2** data is agreement with previously published assignments.[13]
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27. A solution of 2 (1000 mg, 1.33 mmol), 1,3-dicyclohexylcarbodiimide (140 mg, 2.03 mmol), and allylamine (150 mg, 2.33 mmol) in 50 cm^3 of dichloromethane and 1-hydroxybenzotriazole hydrate (225 mg, 1.46 mmol) dissolved in 15 cm^3 of tetrahydrofuran were mixed together and stirred at 0 °C for 1 h. After this time, the reaction mixture was stirred at room temperature for a further 24 h. Then the solvents were distilled under reduced pressure to dryness. The residue was suspended in hexane and filtered off to remove the 1,3-dicyclohexylurea by-product. The filtrate was evaporated under reduced pressure and purified by Dry Column Vacuum Chromatography on silica gel (Fluka type 60) to give **3a** as a colourless solid (893 mg, 85% yield). Pure compound **3a** was dissolved in acetonitrile. The solution was allowed to evaporate at room temperature. After several days the crystals were formed in 57% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm: 6.53 (t, J = 5.71 Hz, 1H), 6.08 (dd, J = 10.74, 2.23 Hz, 1H), 5.94 (dd, J = 10.67, 1.47 Hz,

1H), 5.95–5.86 (m, 1H), 5.21 (qd, J = 17.17, 1.74 Hz, 1H), 5.06 (qd, J = 10.29, 1.58 Hz, 1H), 4.18 (tddd, J = 15.79, 6.49, 4.80, 1.73 Hz, 1H), 4.10 (ddd, J = 9.07, 6.65, 1.21 Hz, 1H), 4.01–3.98 (m, 1H), 3.97 (t, J = 1.89 Hz, 1H), 3.96–3.92 (m, 1H), 3.89 (ddd, J = 9.79, 5.85, 1.79 Hz, 1H), 3.81 (d, J = 8.34 Hz, 1H), 3.79 (q, J = 6.97 Hz, 1H), 3.68 (ddd, J = 9.89, 4.42, 2.62 Hz, 2H), 3.61 (dd, J = 10.88, 3.36 Hz, 1H), 2.98 (qd, J = 9.17, 7.30 Hz, 1H), 2.87 (d, J = 6.59 Hz, 1H), 2.70 (dt, J = 10.75, 4.38 Hz, 1H), 2.60 (td, J = 8.77, 2.77 Hz, 1H), 2.47 (s, 1H), 2.32 (td, J = 12.63, 9.70 Hz, 1H), 2.17 (ddd, J = 12.86, 9.77, 3.37 Hz, 1H), 1.96 (ddd, J = 12.45, 8.88, 3.37 Hz, 1H), 1.92–1.29 (m, 23H, signals are strongly overlapped), 1.28 (s, 3H), 1.25 (d, J = 6.91 Hz, 1H), 1.12 (dd, J = 25.21, 12.86 Hz, 1H), 0.94 (d, J = 6.92 Hz, 1H), 0.92 (d, J = 7.40 Hz, 3H), 0.89 (dd, J = 6.08, 1.42 Hz, 3H), 0.87 (dd, J = 7.50, 1.10 Hz, 3H), 0.87 (d, J = 6.53 Hz, 3H), 0.76 (d, J = 6.93 Hz, 3H), 0.71 (d, J = 6.70 Hz, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2) δ ppm: 213.7, 175.8, 136.1, 133.8, 120.8, 115.1, 106.7, 99.2, 88.8, 79.9, 77.4, 75.7, 74.6, 71.5, 71.0, 65.7, 67.6, 54.8, 48.8, 46.9, 41.9, 40.8, 38.9, 37.1, 36.8, 33.3, 31.0, 30.6, 29.5, 28.7, 26.9, 26.0, 22.6, 22.4, 20.8, 18.4, 17.5, 15.8, 14.9, 14.7, 13.9, 12.2, 11.5, 8.3, 6.6; FT-IR (KBr, cm^{-1}), 3495 ν(O–H), 3430 ν(O–H), 3495 ν(N–H), 1710 ν(C(11)=O), 1655 (amide I band), 1645 ν(C=C, allyl group), 1528 (amide II band); Elemental analysis calcd for $C_{45}H_{75}NO_{10}\cdot C_2H_3N$,: C, 67.92; H, 9.46; N, 3.37; Found: C, 67.81; H, 9.55; N, 3.21. The exemplary spectra are included in the Supplementary data.

28. The micro-organisms used in the tests were the following: Gram-positive cocci: *Staphylococcus aureus* NCTC 4163, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus epidermidis* ATCC 35984, Gram-negative rods: *Escherichia coli* ATCC 10538, *Escherichia coli* ATCC 25922, *Escherichia coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* NCTC 6749, *Pseudomonas aeruginosa* ATCC 27853, *Bordetella bronchiseptica* ATCC 4617 and yeasts-like organisms: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019. The micro-organisms used here were provided by the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland. The antimicrobial activities of studied compounds were performed according to method given in Ref. [27]
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32. Antimicrobial activity was examined in the disc-diffusion and MIC method under standard conditions using Mueller-Hinton II agar medium (Becton Dickinson) for bacteria and RPMI agar with 2% glucose (Sigma) for yeasts, according to CLSI (previously NCCLS) guidelines.[33] The compounds giving some growth inhibition zone in disc-diffusion assay were tested by the twofold serial agar dilution technique to determine their minimal inhibitory concentration (MIC) values.[34] For the disc diffusion method, sterile filter paper discs (9 mm diameter, Whatman No 3 chromatography paper) were dripped with the compound solutions tested (in ethanol) to load 400 μg of a given compound per disc. Dry discs were placed on the surface of an appropriate agar medium. The results (diameter of the growth inhibition zone) were read after 18 h of incubation at 35 °C. For determination of MICs, all compounds were dissolved in DMSO. Concentrations of the agents tested in solid medium ranged from 0.25 to 400 μg/ml. The final inoculum of all organisms studied was 10^4 CFU mL^{-1} (colony forming units per ml). A control test was also performed for DMSO which was found inactive in the culture medium. Minimal inhibitory concentrations were read off after 18 h (for bacteria) and 24 h (for yeasts) of incubation at 35 °C. Ionophore antibiotic—saliomycin A was used as a control for bacteria and fluconazole for yeast.
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