Singly and doubly modified analogues of C20-*epi*-salinomycin: A new group of antiparasitic agents against *Trypanosoma brucei*

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Abstract: Polyether ionophores, with >120 molecules belonging to this group, represent a class of naturally-occurring compounds that exhibit a broad range of pharmacological properties, including promising activity towards a variety of parasites. In this context, salinomycin (**SAL**) seems to be interesting, as this ionophore has been found to be active against parasites that are responsible for a number of human and animal diseases. On the other hand, less explored is the investigation into the anti-parasitic activity of **SAL** derivatives. Recently, we identified C1 amides and esters of **SAL** and its analogue, C20-oxosalinomycin, as promising structures for trypanocidal drug candidates. In search for novel compounds effective against African trypanosomes, the synthetic access to a completely new series of C20-*epi*-salinomycin (compound **2**) analogues is described in this paper. This series includes products obtained *via* derivatisation of either the C1 carboxyl or the C20 hydroxyl of **2**, but also C1/C20 double modified derivatives. The anti-trypanosomal activity as well as the cytotoxic activity of these analogues were evaluated with bloodstream forms of *T. brucei* and human myeloid HL-60 cells, respectively. It was found that the C20 single modified derivatives **8**, **12**, and **18** (C20 decanoate, C20 ethyl carbonate, and C20 allophanate of **2**, respectively) were the most active compounds in selectively targeting bloodstream-form trypanosomes, with 50% growth inhibition (GI_{50}) values of 0.027–0.043 μM and selectivity indices of 165–353. These results indicate that modification at the C20 position of C20-*epi*-salinomycin **2** can provide semi-synthetic products with enhanced trypanocidal activity that could be of great value for the development of new drugs to treat African trypanosomiasis.

1. Introduction

Polyether ionophores represent a very important group of biologically active molecules of natural origin that have been commercially used for decades as veterinary antibiotics in several countries, primarily to suppress the growth of Gram(+) bacteria, but also protozoan parasites responsible for coccidiosis [1–2]. Functionally, ionophores may affect the permeability of the bacterial outer membrane to selected cations, and such perturbation is thought to be the underlining antimicrobial effect of these compounds [3]. In the group of more than 120 structures reported until now, particularly interesting seems to be salinomycin (**SAL**, Scheme 1) because of its relatively broad range of pharmacological properties, which include not only anti-cancer, but also anti-parasitic activities [4].

In screening studies for molecules with selectivity against cancer stem cells (CSCs), **SAL** was identified as the most promising agent at reducing small sub-populations of breast cancer cells with stem-like phenotype, with significantly higher (>100-fold) potency than that of the widely used anti-cancer chemotherapeutic paclitaxel [5]. Importantly, when used at relevant doses (200–250 μg kg^{-1}), **SAL** was well-tolerated by cancer patients, who generally did not experience any long-term acute adverse effects when treated with the ionophore [6]. **SAL** was also found to be effective in killing a series of other cancer cells of different origin, including cancer cell lines resistant to standard chemotherapeutic drugs [7]. Of note is that compounds that exhibit significant activity on cancer cells, very often are also active against parasites [8]. Indeed, using *in vitro* tests, **SAL** has been identified recently as an agent effective against *Trypanosoma brucei* [9], a species of parasitic kinetoplastid which is responsible for African trypanosomiasis in humans (sleeping sickness) and animals (nagana disease) [10–12].

African trypanosomiasis has regularly and repeatedly affected both the economic and cultural development of Central African societies [13]. While the number of sleeping sickness cases reported annually has declined to <1000 in recent years [14], nagana disease still remains a major problem for the rural economy in many endemic African countries. Moreover, the history of African trypanosomiasis has shown that interruption of preventive measures can cause subsequent epidemics of the disease [13]. As the few drugs commonly used for the treatment of African trypanosomiasis are dated, less effective and relatively toxic [15–17], the development of better-tolerated drug candidates is of great importance.

Recently, we have identified various C1 amides and esters of **SAL** and its C20-oxo analogue as possible lead compounds for the development of novel anti-trypanosomal agents [9,18]. The most promising agents in this series inhibited the growth of bloodstream forms of *T. brucei* in the nanomolar concentration range [9,18]. Furthermore, some of the analogues displayed superior trypanocidal activity, which was much higher than that observed for the parent compound **SAL**, with GI_{50} and MIC values comparable to those of suramin, a commonly used medication in the therapy of sleeping sickness [9,18]. In addition to their potent anti-parasitic activity, **SAL** amides and esters obtained *via* derivatisation of the C1 carboxyl group have shown activity against drug-sensitive and drug-resistant cancer cells of various origin, which clearly demonstrates the promising therapeutic potential of this class of compounds, especially as they exhibited high selectivity of action (low toxicity on non-tumour cells) [19–22].

In 2016, Wu and co-workers synthesized C20-*epi*-salinomycin (compound **2**, Scheme 1), together with six of its C20 ester analogues, and evaluated these compounds with respect to their *in vitro* anti-proliferative activity towards colorectal, gastric and triple-negative breast cancer cell lines [23]. Importantly, while **2** exhibited similar activity to that of unmodified **SAL**, its C20-*O*-

acylated derivatives were 2–10 times more effective in killing cancer cells [23]. Two C20-*epi*-esters were also found to be more selective in inhibiting cancer cells than **SAL** [23]. These findings prompted us to examine whether analogues of **2** display also increased trypanocidal activity.

Here, we describe the synthetic access to a variety of *O*-acylated derivatives of **2**, including not only its C20 esters, but also C20 carbonates and C20 carbamates (urethanes), which have been synthesized for the first time (Scheme 1). As **SAL** derivatives with a chemically modified C1 carboxyl group have been shown to exhibit potent activity towards *T. brucei* [9,18], we also decided to obtain the respective C1 amide and ester counterparts of **2**, together with a completely new series of C1/C20 double modified analogues by joining together desirable structural features to produce drug candidates with promising trypanocidal activity (Scheme 1). The *in vitro* trypanocidal and cytotoxic activity of the newly synthesized compounds were evaluated using bloodstream form of *T. brucei* and human myeloid HL-60 cells, respectively. Moreover, the anti-trypanosomal mechanism of action of the most trypanocidal analogues was investigated through cell swelling experiments.

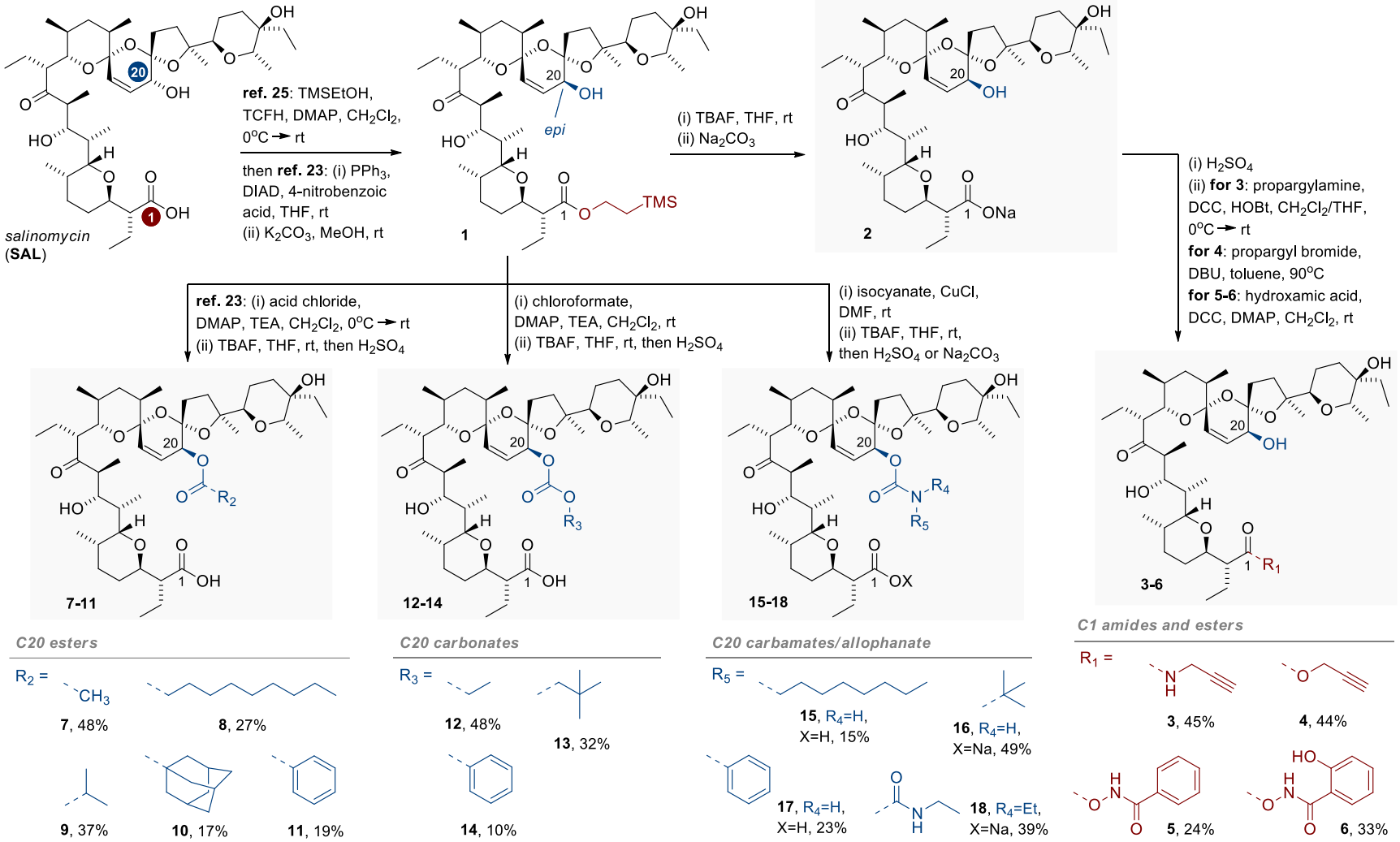
2. Results and discussion

2.1. Analogues design and synthesis

C20-*epi*-salinomycin **2** (Scheme 1) was synthesized according to the protocol reported by Wu and co-workers [23]. Briefly, because of the unfavourable conformation of **SAL** for the Mitsunobu reaction, it was first necessary to ‘mask’ the C1 functionality of the starting material by a relatively bulky protecting group [23]. The reaction between **SAL** and TMSEtOH in the presence of TCFH and DIPEA [24] or DMAP [25] resulted in the formation of C1-protected ester with satisfactory yield. The selective inversion of the C20 absolute configuration was then

smoothly carried out in the Mitsunobu reaction, using 4-nitrobenzoic acid as a nucleophile [23]. As the allylic hydroxyl is more reactive than the other two hydroxyl groups, full regioselectivity towards the hydroxyl group at the C20 position was noted without formation of any C9- and/or C28-substituted side-products. Finally, K_2CO_3 was used to hydrolyse the 4-nitrobenzoyl group at the C20 position to give the key intermediate **1** without affecting the C1 ester moiety [23], while **2** was quantitatively and tracelessly released from its orthogonal masking group with TBAF [24]. The NMR data of product **2** were well in line with those found in the reference literature [23].

In the next step, novel derivatives of **2** were designed and synthesized based on structurally similar C1 amides and esters of **SAL** that exhibited promising trypanocidal activity in our previous studies [9,18], *i.e.* propargyl amide **3**, propargyl ester **4**, and two esters with hydroxamic acids **5** and **6** (Scheme 1). Synthetically, amide **3** was formed in a DCC/HOBt activated reaction with propargylamine (Scheme 1). Depending on the substrate used, the products from the ester series were obtained by two different methods; ester **4** was formed through direct alkylation of carboxylate ions, using propargyl bromide and DBU as effective nucleophilic catalyst, while analogues **5** and **6** were produced *via* the DCC/DMAP-activated esterification with the corresponding hydroxamic acids, *i.e.* benz- and salicylhydroxamic acid, respectively (Scheme 1).

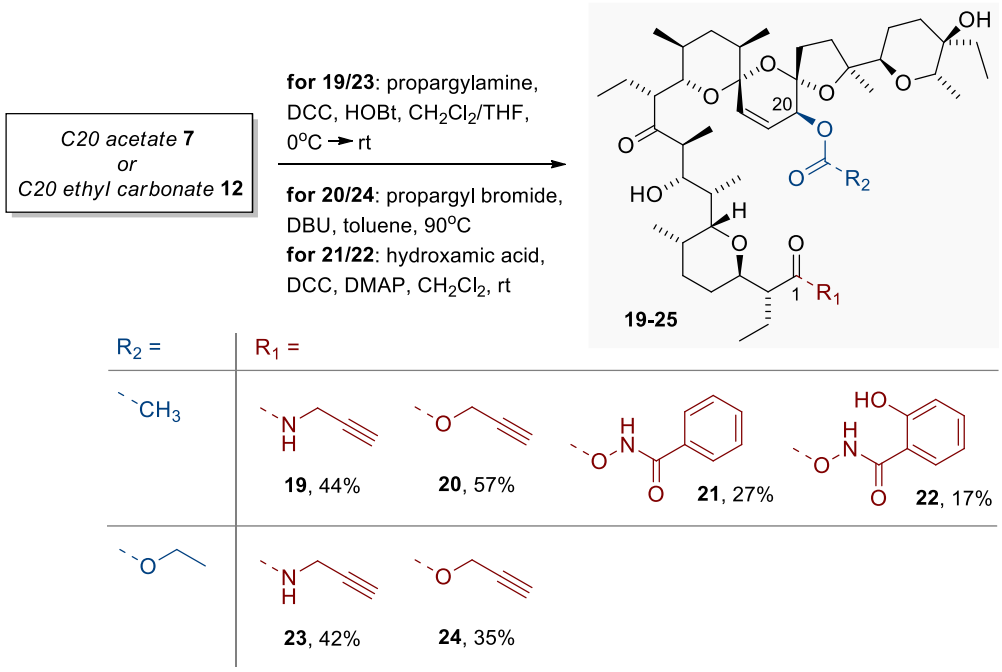


Scheme 1. Synthesis of single modified analogues of C20-*epi*-salinomycin.

Having access to the key intermediate **1**, numerous C20-*O*-acylated analogues of **2** were also obtained (Scheme 1). Firstly, the acylation of **1** with respective acid chlorides in the presence of TEA and excess of DMAP afforded the corresponding C20-*epi*-ester derivatives **7**–**11** in moderate yields (Scheme 1). This group included the literature-known esters **7** and **11** [23] and three other derivatives (**8**–**10**) that were synthesized for the first time which differed in length and arrangement (branching) of the aliphatic substituents. In all cases, the TMSEt masking group could be cleanly cleaved with TBAF [24], and the products were isolated in acid form after washing with aqueous solution of H_2SO_4. Moreover, to widen the structural diversity at the C20 position and to facilitate structure-activity relationship (SAR) studies, we also devised and obtained a completely new series of C20-*epi*-carbonates and C20-*epi*-carbamates (Scheme 1). While C20-*epi*-carbonates **12**–**14** were readily obtained from the reactions with a variety of chloroformates, C20-carbamoylated products **15**–**17** were formed exclusively in reactions with respective isocyanates that required addition of catalytic amounts of CuCl (Scheme 1). Of note is that under the same reaction conditions, treatment of **1** with ethyl isocyanate and CuCl gave the corresponding allophanate **18** (Scheme 1) as the main product. After deprotection with TBAF [24] and further extraction with aqueous solution of H_2SO_4, C20 carbonates and two C20 carbamates of **2** (compound **15** and **17**) were obtained in acid form with the overall yield of 10–48%. However, as carbamate **16** and allophanate **18** were found to undergo decomposition upon acidic extraction, we decided to isolate these products exclusively in the sodium salt form, which was accomplished by washing them with aqueous solution of Na_2CO_3.

Finally, as a continuation of our studies into the synthesis of multiple-modified **SAL** derivatives with improved activity profiles, we also synthesized a completely new series of C1/C20 double modified derivatives of **2** (Scheme 2). According to the procedures for the single modified

analogues (Scheme 1), the C1 carboxyl group of two selected C20-*epi*-*O*-acylated derivatives, *i.e.* C20 acetate **7** and C20 ethyl carbonate **12**, was transformed to the respective amide and ester derivatives **19**–**22** (C20 ester moiety) and **23**–**24** (C20 carbonate moiety) in moderate to good yields (17–57%) (Scheme 2).



Scheme 2. Synthesis of double modified analogues of C20-*epi*-salinomycin.

Purity and structure of the newly obtained analogues of **SAL** were determined based on spectroscopic (FT-IR, NMR) data as well as on spectrometric (ESI MS) data. The NMR and ESI MS spectra of all novel analogues of **2** can be found in the Supplementary material (Figures S1–S63). The positions of the C20-*O*-acyl groups in the structures of each derivative could be unequivocally assigned to a diagnostic downfield shift of the allylic protons relative to the position of the corresponding signal for **2**. The structure of allophanate **18** was determined on the basis of ^{1}H and ^{13}C NMR spectra (Supplementary material, Figures S40–41), together with respective 2D

NMR spectra, *i.e.* ^{1}H-^{13}C HETCOR, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H COSY (Supplementary material, Figures S43–S45).

Briefly, in the ^{13}C NMR spectra of C1 singly modified analogues, the analytical signals of amide and ester groups were observed at 174.6 ppm (for propargyl amide **3**) and in a narrow range of 173.4–174.8 ppm (for esters **4**–**6**), respectively, while the signal of C1 carboxylate of **2** was found at 184.1 ppm. Further, in the ^{1}H NMR spectra, the signal of the highest analytical significance was that of the amide proton in **3**, which appeared at 6.42 ppm (t, J = 4.8 Hz, 1H). With respect to C20-*epi*-*O*-acylated analogues, in the ^{13}C NMR spectra of the newly synthesized analogues **8**–**10**, the most characteristic signals of the ester groups introduced at C20 position were observed in a narrow range of 172.8–176.5 ppm, while for C20-*epi*-carbonates **12**–**14**, C20-*epi*-carbamates **15**–**17** and allophanate **18**, the corresponding *O*-acyl signals were found in the range of 153.1–154.9 ppm, 152.7–156.0 ppm and 155.8 ppm, respectively. For C1/C20 doubly modified derivatives of **2**, the positions of these characteristic signals were insignificantly shifted towards higher or lower ppm values.

2.2. Trypanocidal activity

SAL, C20-*epi*-salinomycin **2** and its derivatives **3**–**24** were assessed for their activity towards *T. brucei* bloodstream forms and HL-60 human myeloid cells *in vitro*, employing the resazurin cell viability test [26–27]. The trypanocidal and cytotoxic activity of the compounds were expressed as MIC values (minimum inhibitory concentration, which is the concentration of a compound that kills all cells; shown in Table S1) and GI_{50} values (50% growth inhibition value, which is the concentration of a compound needed to reduce the cell growth by 50% in comparison with controls; shown in Table 1).

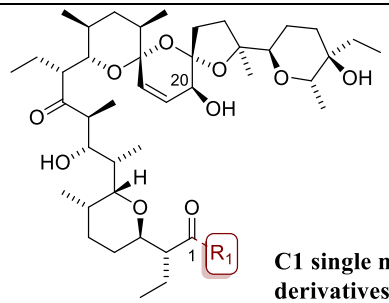
The first thing to mention is that C20-*epi*-salinomycin **2** is 10 times less trypanocidal with respect to GI_{50} and MIC values than **SAL** (Table 1 and Table S1). This finding is remarkable, given that the difference between **2** and **SAL** is just the stereoisomeric configuration of the C20 hydroxyl group. In contrast, no significant difference in cytotoxic activity against human HL-60 cells was found between the two epimers **2** and **SAL**. All synthesized derivatives (**3**–**24**) of **2** showed a concentration-dependent anti-proliferative effect on *T. brucei* bloodstream-form. With the exception of compounds **3**, **4**, **6**, **7**, **13**, **17**, **19**, **20**, **23**, and **24**, all other derivatives displayed higher anti-trypanosomal activity than the parent substance **2**. The most trypanocidal compounds were **8**, **12** and **18** with MIC values of ≤1 μM and mid-nanomolar GI_{50} values. Importantly, these three derivatives showed similar anti-trypanosomal activity as suramin, a medication commonly used in sleeping sickness therapy. With respect to GI_{50} values, most derivatives showed similar or slightly increased cytotoxic activity towards human HL-60 cells than the parent compound **2**. The most trypanocidal compounds **8**, **12** and **18** were also the most cytotoxic compounds. The derivatives **4**, **16**, **19**, and **24** displayed no cytotoxic action against human HL-60 cells (MIC and GI_{50} value >100 μM). All derivatives (except **4**, **16**, **19**, and **24**) displayed the same MIC value of 100 μM for HL-60 cells as compound **2**.

For most derivatives, the selectivity indices (MIC and GI_{50} ratios of cytotoxicity to anti-trypanosomal activity) were ≤100 (Table 1 and Table S1). Only compounds **8**, **12**, **15**, **16**, and **18** had selectivity indices of ≥100 and thus approached the MIC and GI_{50} ratios of suramin. Nevertheless, as suramin does not exhibit any toxicity towards human HL-60 cells, its selectivity still surpasses that of the most trypanocidal compounds.

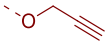
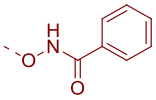
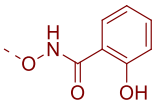
SAR analysis did not reveal many conclusive correlations between the different chemical modifications and trypanocidal activity. For example, the modification pattern of the three most

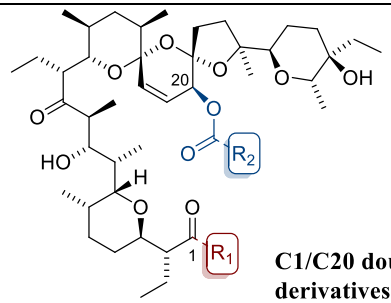
trypanocidal derivatives **8**, **12** and **18** did not have anything in common. However, it was found that C1 propargyl esters (**4**, **20**, and **24**) and C1 propargyl amides (**3**, **19**, and **23**) displayed lower trypanocidal activity than the unmodified compound **2**. Moreover, additional modification of the C20 hydroxyl group (**19**, **20**, **23**, and **24**) had little effect on the trypanocidal action indicating that propargyl esterification and amidation of C1 was responsible for the loss of activity. For instance, C1 propargyl ester or amide modification of compound **12**, one of the three most trypanocidal derivatives, led to a nearly 100-fold reduction of anti-trypanosomal activity of the resulting compounds **19** and **20**. Likewise, a reduction in trypanocidal activity after introduction of the propargyl moiety at the C1 position were previously obtained with **SAL** and its C20-oxo analogue [9,18]. On the other hand, C1 benzhydroxamic acid esters (**5** and **21**) and C1 salicylhydroxamic acid esters (**6** and **22**) showed increased trypanocidal activity. Again, similar observations were previously made with C1 benzhydroxamic and C1 salicylhydroxamic acid esters of **SAL** and C20-oxosalinomycin [18]. Modification of the C20 hydroxyl group with a long aliphatic chain (C20 deconate **8** and C20 octyl carbamate **15**) resulted in derivatives with increased trypanocidal activity. Whether the increased anti-trypanosomal activity was due to increased lipophilicity brought by the aliphatic chain, remains to be shown.

Table 1. GI_{50} values and ratios of salinomycin (**SAL**), C20-*epi*-salinomycin (**2**) and its analogues (**3–24**) for *T. brucei* and HL-60 cells.

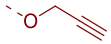
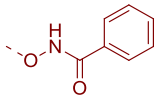
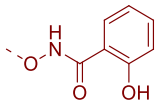


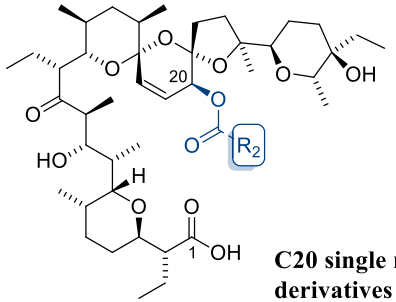
C1 single modified derivatives

No.	R_1 =	*T. brucei* GI_{50} (μM)[a]	HL-60 GI_{50} (μM)[a]	Selectivity GI_{50} ratio[b]
2	- -ONa	2.97 ± 0.08	44.8 ± 12.9	15.1
3		8.49 ± 2.74	39.3 ± 5.1	4.6
4		26.9 ± 1.6	>100	>3.7
5		0.32 ± 0.01	9.54 ± 0.44	29.8
6		2.81 ± 0.07	33.2 ± 2.5	11.8



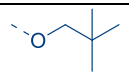
C1/C20 double modified derivatives

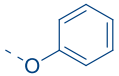
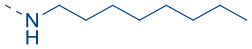
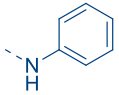
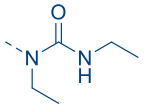
No.	R_1 =	R_2 =	*T. brucei* GI_{50} (μM)[a]	HL-60 GI_{50} (μM)[a]	Selectivity GI_{50} ratio[b]
19		- -CH_3	20.9 ± 1.7	39.8 ± 7.5	1.9
23			13.8 ± 4.0	61.8 ± 4.4	4.5
20		- -CH_3	22.4 ± 2.0	>100	>4.5
24			15.0 ± 4.9	>100	>6.7
21		- -CH_3	0.47 ± 0.22	19.1 ± 1.1	40.6
22		- -CH_3	0.59 ± 0.22	20.3 ± 9.5	34.4



C20 single modified derivatives

No.	R_2 =	*T. brucei* GI_{50} (μM)[a]	HL-60 GI_{50} (μM)[a]	Selectivity GI_{50} ratio[b]
7	- -CH_3	2.48 ± 0.19	52.5 ± 14.8	21.2

No.	R_2 =	*T. brucei* GI_{50} (μM)[a]	HL-60 GI_{50} (μM)[a]	Selectivity GI_{50} ratio[b]
13		21.6 ± 6.3	42.4 ± 4.1	2.0

8		0.036 ± 0.007	5.97 ± 0.40	165	**14**		0.22 ± 0.03	23.4 ± 4.1	106
9		0.24 ± 0.04	21.6 ± 2.1	90.0	**15**		0.20 ± 0.03	24.6 ± 3.4	123
10		0.34 ± 0.05	18.7 ± 6.3	55.0	**16**[c)]		0.32 ± 0.04	>100	>312
11		0.20 ± 0.06	13.5 ± 1.7	67.5	**17**		3.15 ± 0.16	57.2 ± 7.0	18.2
12		0.043 ± 0.010	12.6 ± 0.4	293	**18**[c)]		0.027 ± 0.002	9.54 ± 0.6	353

Reference controls

No.	*T. brucei* GI$_{50}$ (µM)[a)]	HL-60 GI$_{50}$ (µM)[a)]	Selectivity GI$_{50}$ ratio[b)]	**No.**	*T. brucei* GI$_{50}$ (µM)[a)]	HL-60 GI$_{50}$ (µM)[a)]	Selectivity GI$_{50}$ ratio[b)]
SAL	0.30 ± 0.08	50.5 ± 3.9	168	**Suramin**	0.058 ± 0.026	>100	>1724

[a)] Data shown are mean values ± SD of three independent experiments; [b)] GI$_{50}$ ratio = GI$_{50}$(HL-60)/GI$_{50}$(*T. brucei*); [c)] Carbamate **16** and allophanate **18** in the sodium salt form.

Of note is that **SAL** derivatives that have been identified as potent trypanocidal agents, generally also showed increased ionophoretic activity [9,18]. To see, whether the reduced and increased anti-trypanosomal activity of C20-*epi*-salinomycin **2** and of the most trypanocidal derivatives **8**, **12**, and **18**, respectively, was linked to their ionophoretic activity, swelling experiments were performed. Upon exposure to ionophoretic active compounds, the cell volume of bloodstream form trypanosomes usually increases, which can be determined by light scattering measurements [9,18,26]. In contrast to **SAL**, C20-*epi*-salinomycin **2** did not induce significant swelling in trypanosomes (Figure 1). This result is in agreement with the finding that **2** was about 10-times less trypanocidal than **SAL** (Table 1 and Table S1) and with previous observations that compounds with MIC values of ≥10 µM and GI_{50} values of ≥3 µM display much lower ionophoretic activity [9]. Compared to **SAL**, the derivatives **8**, **12**, and **18** produced slightly enhanced swelling in trypanosomes. This result confirms previous findings that compounds with lower MIC and GI_{50} values than **SAL** generally display increased ionophoretic activity [9,18].

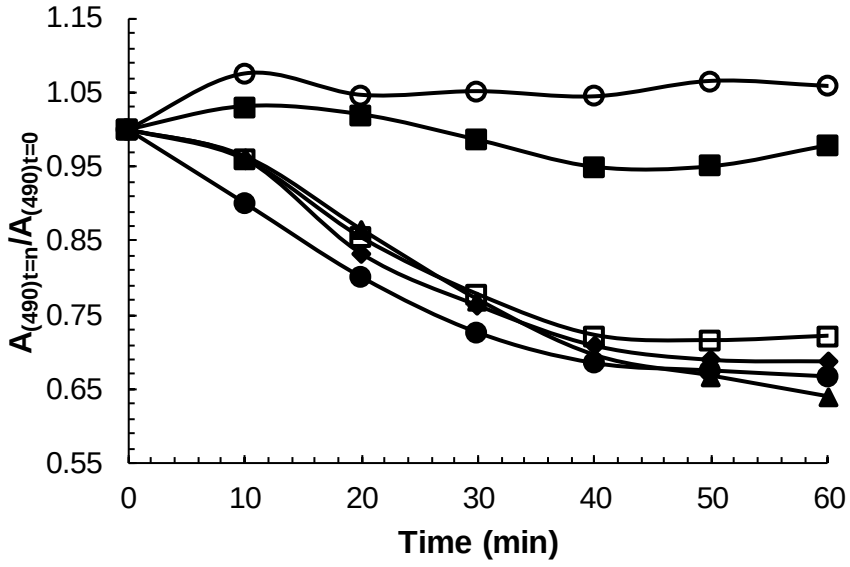


Figure 1. Effect of C20-*epi*-salinomycin analogues on cell swelling of bloodstream forms of *T. brucei*. Trypanosomes (5×10^7 mL^{-1}) were exposed to 100 µM ionophoretic active compounds in Baltz medium in the presence of 0.9%

DMSO. The absorbance at 490 nm of the culture was measured every 10 min. Open circles, DMSO control; open squares, **SAL** (salinomycin); closed squares, **2** (C20-*epi*-salinomycin); closed triangles, **8** (C20 decanoate of C20-*epi*-salinomycin); closed diamonds, **12** (C20 ethyl carbonate of C20-*epi*-salinomycin); closed circles, **18** (C20 allophanate of C20-*epi*-salinomycin). An increase in absorbance is reflected by a decrease in absorbance. For reasons of simplicity, only average values of three independent experiments are shown. The standard deviations varied between 1.1 and 11.4 percentage points.

3. Conclusions

Taken together, a series of analogues of C20-*epi*-salinomycin (**2**) was synthesized. It included products obtained by chemical modification at either C1 or C20 position of **2**, but also C1/C20 doubly modified derivatives. All derivatives were assessed for their anti-trypanosomal activity and selectivity using *in vitro* experiments. More than half of the compounds showed higher activity towards *T. brucei* than the parent substance **2**, but only three (**8**, **12**, and **18**) displayed enhanced anti-trypanosomal action compared with **SAL**. However, six analogues (**8**, **12**, **14**, **15**, **16**, and **18**) meet the activity criteria for hit compounds for *T. brucei*, which are GI_{50} <1 μM and selectivity >100 [28]. Interestingly, all six compounds were C20 single modified derivatives indicating that modification at the C20 position may be the rationale for future drug candidate development.

4. Experimental

4.1. General procedures

All commercially available reagents and solvents were purchased from two independent sources (Merck (Germany) or Trimen Chemicals S.A. (Poland)) and used in the experiments without further purification. Detailed description of general procedures, used equipment (NMR

spectrometer, FT-IR spectrophotometer, mass spectrometer), measurement parameters and software can be found either in the Supplementary material or in the reference literature [18–19]. The ^{1}H and ^{13}C NMR signals of allophanate **18** were assigned using the gradient-enhanced version of the 2D experiments (^{1}H-^{13}C HETCOR, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H COSY) shown in the Supplementary material (Figures S43–S45). The 2D spectra were recorded using standard pulse sequences from Bruker pulse-sequence libraries.

4.2. Synthesis

SAL in its sodium salt form was isolated in gram quantities from commercially available veterinary premix SACOX®, according to previous protocols [21–22]. To obtain the free acid form of **SAL**, the isolated ionophore in its sodium salt form was dissolved in CH_2Cl_2 and extracted 2–3 times with aqueous sulphuric acid (pH = 1.0) by vigorously mixing the organic and aqueous layers. The collected organic layers containing **SAL** were thoroughly washed once with water, and then evaporated *in vacuo* giving sodium-free **SAL** as a clear oil. The oil was easily transformed into a white amorphous solid after thrice evaporation with *n*-pentane to dryness. The spectroscopic data of **SAL** were in line with previously published data [29].

C20-*epi*-salinomycin **2**, together with its two C20 ester derivatives (compound **7** and **11**, Scheme 1) were re-synthesized following the protocol published by Wu and co-workers [23]. The NMR data of **2** as well as its analogues **7** and **11** were in line with those found in the reference literature.

*4.2.1. Synthesis of C1 propargyl amide of C20-epi-salinomycin (analogue **3**)*

Initially, the sodium salt of C20-*epi*-salinomycin **2** was dissolved in CH_2Cl_2 and quantitatively transformed into its free acid form by twice extraction with sulphuric acid (pH = 1.0) and subsequent washing of the combined organic layers with water, and evaporation of the organic solvent *in vacuo*. Then, to a stirred solution of **2** (100 mg, 0.13 mmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (10 mL) at 0 °C, the following reagents were added: DCC (32 mg, 0.16 mmol, 1.2 equiv.), HOBt (10 mg, 0.07 mmol, 0.5 equiv., dissolved in 3 mL of anhydrous THF) and propargylamine (18 mg, 0.33 mmol, 2.5 equiv.). The temperature of the reaction mixture was raised to room temperature, and stirring was continued for 48 h. Then, the mixture was concentrated *in vacuo* and purified chromatographically on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) to give the pure product **3** (45% yield) as a clear oil. After thrice evaporation to dryness with *n*-pentane, the oily product was completely converted into a white amorphous solid. The ^{1}H and ^{13}C NMR spectra of the amide **3** can be found in the Supplementary material (Figures S1–S2).

Yield: 47 mg, 45%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.42 (t, *J* = 5.5 Hz, 1H), 5.89 (dd, *J* = 10.1, 1.6 Hz, 1H), 5.42 (dd, *J* = 10.1, 2.3 Hz, 1H), 4.36 (ddd, *J* = 17.8, 5.9, 2.3 Hz, 1H), 4.17–4.13 (m, 2H), 4.11–4.06 (m, 2H), 3.95 (dd, *J* = 10.0, 5.1 Hz, 1H), 3.85 (d, *J* = 8.4 Hz, 2H), 3.83–3.79 (m, 1H), 3.68 (dd, *J* = 15.1, 6.8 Hz, 2H), 3.12 (d, *J* = 5.0 Hz, 1H), 2.97 (dt, *J* = 14.5, 7.4 Hz, 1H), 2.35–2.24 (m, 4H), 2.14 (t, *J* = 2.5 Hz, 1H), 2.00–0.50 (m, 52H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.9, 174.6, 131.0, 128.9, 109.7, 99.9, 86.6, 80.8, 77.6, 75.2, 74.1, 71.2, 70.8, 70.6, 69.9, 69.2, 57.2, 49.2, 48.6, 38.9, 38.6, 36.1, 36.0, 33.2, 31.1, 30.8, 30.5, 29.1, 28.9, 28.2, 26.4, 25.9, 22.6, 22.2, 21.3, 20.3, 18.1, 15.6, 14.5, 13.8, 13.3, 11.8, 11.3, 7.4, 6.3 ppm; FT-IR (KBr tablet): 3447 (br, m), 3316 (m), 2968 (s), 2939 (s), 2877 (m), 2124 (w), 1720 (m), 1659

(w), 1533 (m), 1454 (m), 1387 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{45}H_{73}NNaO_{10}^+$ 810.5; Found 811.

*4.2.2. Synthesis of C1 propargyl ester of C20-epi-salinomycin (analogue **4**)*

To a solution of sodium-free **2** (prepare as described above; 100 mg, 0.13 mmol, 1.0 equiv.) in anhydrous toluene (10 mL), DBU (24 mg, 0.16 mmol, 1.2 equiv.) and propargyl bromide (~80% in toluene; 46 mg, 0.39 mmol, 3.0 equiv.) were added under stirring and the solution was heated at 90 °C for 6 h. The solution was then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-hexane) gave the pure product **4** (44% yield) as a clear oil. After thrice evaporation to dryness with *n*-pentane, the oily product was completely converted into a white amorphous solid. The ^1H and ^{13}C NMR spectra of ester **4** can be found in the Supplementary material (Figures S4–S5).

Yield: 46 mg, 44%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 5.84 (dd, *J* = 10.1, 1.6 Hz, 1H), 5.41 (dd, *J* = 10.1, 2.5 Hz, 1H), 5.04 (dd, *J* = 15.8, 2.4 Hz, 1H), 4.84 (dd, *J* = 15.8, 2.5 Hz, 1H), 4.16–4.14 (m, 1H), 4.06 (dd, *J* = 11.0, 5.8 Hz, 1H), 3.96 (dd, *J* = 9.1, 5.4 Hz, 1H), 3.90 (d, *J* = 1.1 Hz, 1H), 3.87–3.78 (m, 2H), 3.71 (dd, *J* = 10.8, 3.3 Hz, 1H), 3.62 (dd, *J* = 9.8, 1.6 Hz, 1H), 3.11–2.98 (m, 2H), 2.74 (d, *J* = 5.5 Hz, 1H), 2.69 (ddd, *J* = 8.8, 4.8, 1.7 Hz, 1H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.42 (s, 1H), 2.39–2.33 (m, 1H), 2.32–2.26 (m, 1H), 2.26–2.19 (m, 2H), 2.00–0.50 (m, 50H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.0, 174.8, 130.5, 129.2, 109.7, 99.9, 86.5, 78.2, 77.3, 76.8, 74.80, 74.75, 74.1, 71.7, 70.8, 69.5, 69.3, 56.6, 52.6, 48.4, 48.2, 38.8, 36.3, 35.9, 33.2, 30.9, 30.7, 30.5, 29.2, 28.0, 26.2, 25.9, 22.7, 22.2, 20.9, 19.6, 18.1, 15.7, 14.5, 13.8, 13.3, 11.7, 10.8, 7.2, 6.3 ppm; FT-IR (KBr tablet): 3550 (br, m), 3438 (m), 3311 (m), 2965 (s), 2939 (s), 2877

(m), 2124 (w), 1723 (s), 1624 (w), 1460 (m), 1387 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{45}H_{72}NaO_{11}^+$ 811.5; Found 811.9.

*4.2.3. General procedure for preparation of C1 esters of C20-epi-salinomycin with hydroxamic acids (analogues **5** and **6**)*

To a solution of sodium-free **2** (prepared as described above; 1.0 equiv.) in anhydrous CH_2Cl_2, DCC (2.0 equiv.) and DMAP (2.0 equiv.) were introduced under stirring at ambient temperature. Then, the respective hydroxamic acid (5.0 equiv.) was added in one portion. The resulting solutions were stirred for 48 h, and subsequently diluted with CH_2Cl_2 and washed with saturated NH_4Cl. The organic phases of the reaction mixtures were separated and concentrated *in vacuo* to give clear oils. Purification on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) gave the pure products **5** and **6** (24–33% yield) as clear oils. After thrice evaporation to dryness with *n*-pentane, the oily products were completely converted into white amorphous solids. The ^1H and ^{13}C NMR spectra of esters **5** and **6** can be found in the Supplementary material (Figures S7–S8 and Figures S10–S11, respectively).

*C1 benzhydroxamic acid ester of C20-epi-salinomycin **5**:* Yield: 28 mg, 24%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 11.09 (s, 1H), 8.12 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.47–7.40 (m, 1H), 7.34 (dd, *J* = 10.4, 4.7 Hz, 2H), 6.22 (d, *J* = 10.6 Hz, 1H), 6.17–6.11 (m, 1H), 4.07 (dd, *J* = 11.0, 6.2 Hz, 1H), 4.01 (t, *J* = 6.5 Hz, 1H), 3.79 (d, *J* = 3.5 Hz, 1H), 3.75–3.69 (m, 1H), 3.67 (d, *J* = 10.1 Hz, 2H), 3.41 (dd, *J* = 11.9, 2.2 Hz, 1H), 3.12 (td, *J* = 11.0, 3.7 Hz, 1H), 2.92–2.83 (m, 1H), 2.50 (d, *J* = 9.6 Hz, 1H), 2.12 (ddd, *J* = 12.5, 11.5, 8.9 Hz, 1H), 2.00–0.50 (m, 56H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.5, 173.4, 165.4, 131.9, 131.1, 130.6, 128.2, 128.1, 124.4, 108.9,

98.5, 89.3, 79.8, 74.6, 73.4, 72.0, 70.9, 68.4, 66.0, 54.0, 46.7, 46.3, 39.3, 38.3, 36.3, 33.9, 32.4, 31.0, 30.6, 29.4, 28.1, 26.5, 25.8, 25.6, 24.9, 23.2, 21.8, 19.8, 17.1, 16.5, 14.4, 14.3, 14.2, 11.8, 10.9, 7.6, 6.5 ppm; FT-IR (KBr tablet): 3482 (br, m), 3307 (br, m), 2968 (s), 2930 (s), 2875 (m), 1790 (s), 1726 (s), 1697 (s), 1604 (w), 1579 (w), 1504 (w), 1463 (s), 1378 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{75}NNaO_{12}^+$ 892.5; Found 893.

C1 salicylhydroxamic acid ester of C20-epi-salinomycin ***6****:* Yield: 39 mg, 33%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 11.55 (s, 1H), 11.32 (s, 1H), 8.17 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.40 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 6.97 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.87–6.81 (m, 1H), 5.80 (dd, *J* = 10.1, 2.2 Hz, 1H), 5.41 (dd, *J* = 10.1, 2.1 Hz, 1H), 4.18 (dd, *J* = 10.4, 5.0 Hz, 2H), 4.05 (s, 1H), 3.86 (d, *J* = 11.4 Hz, 1H), 3.82 (q, *J* = 6.9 Hz, 1H), 3.73 (dd, *J* = 10.6, 1.6 Hz, 1H), 3.63 (d, *J* = 11.3 Hz, 2H), 3.22 (td, *J* = 10.9, 3.8 Hz, 1H), 3.06 (dt, *J* = 14.8, 7.4 Hz, 1H), 2.84–2.78 (m, 1H), 2.44 (d, *J* = 5.1 Hz, 1H), 2.32–2.29 (m, 1H), 2.22–2.17 (m, 3H), 2.30–0.50 (m, 51H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 218.4, 173.5, 168.8, 161.4, 134.6, 131.0, 129.5, 127.5, 118.7, 118.0, 111.9, 109.7, 99.5, 86.5, 74.5, 73.9, 72.0, 70.8, 69.0, 68.6, 56.4, 49.3, 47.1, 38.8, 35.9, 35.7, 33.9, 32.7, 31.4, 31.2, 30.5, 29.1, 27.9, 26.4, 25.5, 24.9, 23.1, 22.1, 20.7, 19.9, 17.9, 15.6, 14.5, 13.5, 13.2, 11.6, 11.0, 7.1, 6.3 ppm; FT-IR (KBr tablet): 3450 (br, m), 3327 (br, m), 2968 (s), 2933 (s), 2877 (m), 1793 (m), 1764 (m), 1700 (m), 1653 (m), 1627 (m), 1609 (m), 1586 (m), 1513 (m), 1467 (m), 1373 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{75}NNaO_{13}^+$ 908.5; Found 909.

4.2.4. General procedure for preparation of C20 esters of C20-epi-salinomycin (analogues ***7****–****11****)*

To a stirred solution of C20-*epi*-salinomycin intermediate **1** (1.0 equiv.) in anhydrous CH_2Cl_2 at 0 °C, the following reagents were added: TEA (6.0 equiv.), an excess of DMAP, and the corresponding acyl chloride (3.0 equiv.) diluted in anhydrous CH_2Cl_2, which was added drop by drop over 30 seconds. The reaction mixtures were warmed to room temperature, stirred overnight, and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-hexane) gave each intermediate ester product as a clear oil. In the next step, to a stirred solution of each intermediate product in THF at ambient temperature, TBAF (3.0 equiv., 1.0 M in THF) was introduced dropwise over 30 seconds. The resulting slightly yellowish solutions were stirred until the starting materials were completely consumed (TLC control) and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) provided the deprotected products as mixtures of their acid and salt forms. The product mixtures were dissolved again in CH_2Cl_2, washed with sulphuric acid (pH = 1.0), and extracted with water. The organic layers of the reaction mixtures were separated, and evaporated thrice from *n*-pentane to give the pure products **7**–**11** (17–48% yield) as white amorphous solids. The ^{1}H and ^{13}C NMR spectra of the newly synthesized esters **8**, **9** and **10** can be found in the Supplementary material (Figures S13–S14, Figures S16–S17 and Figures S19–S20, respectively).

*C20 decanoate of C20-epi-salinomycin **8**:* Yield: 70 mg, 27%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.34 (d, *J* = 10.6 Hz, 1H), 6.18 (dd, *J* = 10.6, 5.8 Hz, 1H), 4.94 (d, *J* = 5.8 Hz, 1H), 4.12 (d, *J* = 10.0 Hz, 1H), 3.96 (dd, *J* = 11.0, 5.3 Hz, 1H), 3.89 (d, *J* = 10.3 Hz, 1H), 3.84–3.75 (m, 1H), 3.66–3.58 (m, 3H), 2.92–2.83 (m, 1H), 2.78– 2.69 (m, 1H), 2.58 (dd, *J* = 11.7, 9.7 Hz, 1H), 2.22 (td, *J* = 7.4, 2.7 Hz, 2H), 2.00–0.50 (m, 72H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.0, 177.8, 172.8, 127.9, 123.6, 105.8, 99.0, 89.8, 76.5, 75.3, 74.9, 73.7, 71.5, 71.1, 68.3, 66.6,

56.1, 49.8, 49.0, 40.2, 39.1, 36.8, 36.4, 34.4, 32.7, 31.8, 31.3, 30.0, 29.6, 29.4, 29.20, 29.18, 29.1, 27.9, 26.3, 25.6, 24.9, 22.8, 22.6, 22.0, 21.9, 19.9, 17.9, 16.6, 14.2, 14.1, 13.2, 12.9, 12.0, 11.0, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3503 (br, m), 2959 (s), 2927 (s), 2880 (m), 1735 (s), 1714 (s), 1648 (w), 1460 (s), 1381 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{52}H_{88}NaO_{12}^+$ 927.6; Found 928.

C20 isobutyrate of C20-epi-salinomycin ***9****:* Yield: 79 mg, 37%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 6.34 (d, *J* = 10.6 Hz, 1H), 6.16 (dd, *J* = 10.6, 5.8 Hz, 1H), 4.95 (d, *J* = 5.8 Hz, 1H), 4.12 (dd, *J* = 10.2, 1.5 Hz, 1H), 3.96 (dd, *J* = 11.0, 5.6 Hz, 1H), 3.89 (d, *J* = 10.3 Hz, 1H), 3.80 (d, *J* = 6.9 Hz, 1H), 3.62 (ddd, *J* = 9.9, 7.3, 2.8 Hz, 2H), 2.87 (td, *J* = 10.9, 3.7 Hz, 1H), 2.77–2.71 (m, 1H), 2.60 (dd, *J* = 11.0, 2.5 Hz, 1H), 2.46 (pd, *J* = 7.0, 0.7 Hz, 1H), 2.05 (dt, *J* = 9.1, 2.3 Hz, 1H), 2.00–0.50 (m, 61H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.1, 177.8, 175.9, 127.9, 123.6, 105.8, 99.0, 89.83 76.5, 75.3, 74.9, 73.7, 71.5, 71.1, 68.3, 66.4, 56.1, 49.8, 49.0, 40.2, 39.1, 36.8, 36.4, 34.1, 32.7, 31.2, 30.1, 29.6, 27.9, 26.3, 25.7, 22.8, 22.0, 19.8, 18.9, 18.7, 17.9, 16.6, 14.2, 13.2, 12.9, 12.0, 11.0, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3503 (br, m), 2959 (s), 2880 (m), 1741 (m), 1709 (m), 1647 (w), 1460 (m), 1387 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{46}H_{76}NaO_{12}^+$ 843.5; Found 844.

C20-adamantane-1-carboxylate of C20-epi-salinomycin ***10****:* Yield: 44 mg, 17%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 6.35 (d, *J* = 10.7 Hz, 1H), 6.14 (dd, *J* = 10.6, 5.8 Hz, 1H), 4.96 (d, *J* = 5.9 Hz, 1H), 4.12 (d, *J* = 10.3 Hz, 1H), 3.96 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.90 (d, *J* = 10.2 Hz, 1H), 3.82–3.77 (m, 1H), 3.67–3.60 (m, 3H), 2.87 (td, *J* = 10.9, 3.9 Hz, 1H), 2.74 (dd, *J* = 10.2, 7.1 Hz, 1H), 2.63–2.57 (m, 1H), 2.30–0.50 (m, 70H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ

215.2, 177.9, 176.5, 127.8, 123.6, 105.9, 99.0, 89.8, 75.2, 74.9, 73.6, 71.5, 71.2, 68.3, 66.2, 56.1, 49.9, 49.1, 40.7, 40.3, 39.0, 38.7, 36.9, 36.5, 32.7, 31.2, 30.5, 30.1, 29.6, 27.9, 26.3, 25.7, 22.8, 22.0, 19.9, 18.3, 18.0, 16.9, 16.5, 15.9, 14.2, 13.8, 13.3, 13.1, 12.9, 12.1, 11.9, 11.0, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3503 (br, m), 2962 (s), 2936 (s), 2857 (m), 1726 (s), 1709 (s), 1460 (m), 1378 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{53}H_{84}NaO_{12}^+$ 935.6; Found 936.

*4.2.5. General procedure for preparation of C20 carbonates of C20-epi-salinomycin (analogues **12**–**14**)*

To a stirred solution of C20-*epi*-salinomycin intermediate **1** (1.0 equiv.) in anhydrous CH_2Cl_2 at ambient temperature, the following reagents were added: TEA (6.0 equiv.), an excess of DMAP and the corresponding chloroformate (4.0 equiv.) diluted in anhydrous CH_2Cl_2, which was added drop by drop over 30 seconds. The reaction mixtures were stirred overnight at ambient temperature, and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-hexane) gave the intermediate carbonate products as clear oils. In the next step, to a stirred solution of each intermediate product in THF at ambient temperature, TBAF (3.0 equiv., 1.0 M in THF) was introduced dropwise over 30 seconds. The resulting slightly yellowish solutions were stirred until the starting materials were completely consumed (TLC control) and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) provided the deprotected products as mixtures of their acid and salt forms. The product mixtures were dissolved again in CH_2Cl_2, washed with sulphuric acid (pH = 1.0), and extracted with water. The organic layers of the reaction mixtures were separated, and evaporated thrice from *n*-pentane to give the pure products **12**–**14** (10–48% yield) as white amorphous solids. The ^1H and ^{13}C NMR spectra of the newly synthesized carbonates **12**, **13** and

14 can be found in the Supplementary material (Figures S22–S23, Figures S25–S26 and Figures S28–S29, respectively).

*C20 ethyl carbonate of C20-epi-salinomycin **12**:* Yield: 42 mg, 48%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (403 MHz, CD_2Cl_2) δ 6.35 (d, *J* = 10.6 Hz, 1H), 6.14 (dd, *J* = 10.6, 5.6 Hz, 1H), 4.78 (d, *J* = 5.7 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 4.05 (dd, *J* = 10.2, 1.4 Hz, 1H), 3.92 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.86 (d, *J* = 10.2 Hz, 1H), 3.79 (dd, *J* = 13.9, 6.9 Hz, 1H), 3.58 (ddd, *J* = 7.4, 5.3, 2.8 Hz, 2H), 2.88 (td, *J* = 10.7, 4.0 Hz, 1H), 2.78–2.69 (m, 1H), 2.60 (dd, *J* = 10.8, 2.0 Hz, 1H), 2.25–0.50 (m, 59H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 215.2, 177.9, 154.9, 129.1, 123.4, 106.2, 99.3, 90.2, 77.1, 75.9, 75.2, 74.1, 71.9, 71.3, 70.0, 68.8, 64.4, 56.2, 49.8, 49.1, 40.5, 39.4, 36.8, 36.6, 33.1, 32.0, 30.1, 29.9, 28.4, 26.6, 26.0, 23.1, 22.2, 20.3, 18.0, 16.7, 16.6, 14.43, 14.42, 13.3, 13.2, 12.1, 11.2, 7.0, 6.6 ppm; FT-IR (KBr tablet): 3491 (br, m), 2965 (s), 2939 (s), 2878 (s), 1743 (s), 1717 (s), 1648 (m), 1568 (m), 1461 (s), 1376 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{45}H_{74}NaO_{13}^+$ 845.5; Found 846.

*C20 neopentyl carbonate of C20-epi-salinomycin **13**:* Yield: 66 mg, 32%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.39 (d, *J* = 10.6 Hz, 1H), 6.26 (dd, *J* = 10.6, 5.7 Hz, 1H), 4.76 (d, *J* = 5.7 Hz, 1H), 4.13 (d, *J* = 10.1 Hz, 1H), 4.01–3.94 (m, 1H), 3.91–3.85 (m, 3H), 3.83–3.79 (m, 1H), 3.74 (d, *J* = 10.3 Hz, 1H), 3.69–3.60 (m, 3H), 2.93–2.81 (m, 1H), 2.78–2.68 (m, 1H), 2.60 (d, *J* = 9.7 Hz, 1H) 2.30–0.50 (m, 63H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.0, 177.9, 154.8, 128.8, 123.1, 105.6, 99.1, 90.0, 76.5, 75.2, 74.9, 73.8, 71.6, 71.1, 69.7, 68.3, 56.0, 55.9, 49.8, 49.1, 40.2, 39.3, 36.7, 36.4, 32.7, 31.6, 31.3, 29.9, 29.6, 28.0, 26.2, 25.7, 22.8, 22.0, 19.9, 18.0, 16.5, 16.4, 15.7, 14.2, 13.2, 12.9, 12.0, 11.0, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3506 (br, m), 2962 (s),

2939 (s), 2877 (m), 1755 (s), 1709 (s), 1633 (w), 1457 (m), 1387 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{48}H_{80}NaO_{13}^+$ 887.5; Found 888.

C20 phenyl carbonate of C20-epi-salinomycin ***14****:* Yield: 21 mg, 10%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 7.39–7.33 (m, 2H), 7.25–7.19 (m, 1H), 7.15–7.11 (m, 2H), 6.45 (d, *J* = 10.7 Hz, 1H), 6.28 (dd, *J* = 10.6, 5.8 Hz, 1H), 4.91 (d, *J* = 5.8 Hz, 1H), 4.13 (dd, *J* = 8.7, 4.2 Hz, 1H), 3.97 (dd, *J* = 10.6, 5.1 Hz, 1H), 3.92 (d, *J* = 10.3 Hz, 1H), 3.83 (d, *J* = 6.4 Hz, 1H), 3.68 (dd, *J* = 10.2, 3.2 Hz, 1H), 3.65–3.61 (m, 1H), 2.88 (td, *J* = 10.9, 3.8 Hz, 1H), 2.78–2.70 (m, 1H), 2.62–2.55 (m, 1H), 2.30–2.19 (m, 1H), 2.16–2.04 (m, 2H), 2.00–0.50 (m, 53H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.9, 177.8, 153.1, 151.1, 129.5, 129.4, 126.0, 122.4, 121.1, 105.4, 99.0, 90.2, 76.5, 75.2, 74.9, 73.8, 71.6, 71.2, 70.7, 68.3, 56.0, 49.7, 49.0, 40.1, 39.2, 36.8, 36.4, 32.7, 31.3, 29.9, 29.6, 28.0, 26.3, 25.7, 22.7, 22.1, 19.9, 18.0, 16.5, 16.3, 14.2, 13.3, 12.9, 12.0, 11.0, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3491 (br, m), 2971 (s), 2933 (s), 2880 (s), 1764 (s), 1712 (s), 1644 (w), 1597 (w), 1568 (w), 1466 (m), 1387 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{49}H_{74}NaO_{13}^+$ 893.5; Found 894.

4.2.6. General procedure for preparation of C20 carbamates and C20 allophanate of C20-epi-salinomycin (analogues ***15****–****18****)*

To a stirred solution of C20-*epi*-salinomycin intermediate **1** (1.0 equiv.) in anhydrous DMF at ambient temperature, the respective isocyanate (2.0 equiv.) was added drop by drop over 30 seconds, followed by the addition of one spatula tip-full of anhydrous, freshly ground CuCl in one portion. The greenish reaction mixture was stirred for three days at ambient temperature, and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-

hexane) gave the intermediate carbamate/allophanate products as clear oils. In the next step, to a stirred solution of each intermediate product in THF at ambient temperature, TBAF (3.0 equiv., 1.0 M in THF) was introduced dropwise over 30 seconds. The resulting slightly yellowish solutions were stirred until the starting materials were completely consumed (TLC control) and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) provided the deprotected products as mixtures of their acid and salt forms. The product mixtures were dissolved again in CH_2Cl_2, washed with aqueous solution of Na_2CO_3 (0.1 M) in the case of **16** and **18**, or with sulphuric acid (pH = 1.0) in the case of **15** and **17**, and extracted with water. The organic layers of the reaction mixtures were separated, and evaporated thrice from *n*-pentane to give the pure products **15**–**18** (15–49% yield) as white amorphous solids. The NMR spectra of the newly synthesized carbamates **15–17**, and the newly synthesized allophanate **18** can be found in the Supplementary material (Figures S31–S32, Figures S34–S35, Figures S37–S38, Figures S40–S41 and Figures S43–S45, respectively).

*C20 octyl carbamate of C20-epi-salinomycin **15**:* Yield: 33 mg, 15%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.11 (dd, *J* = 10.9, 2.6 Hz, 1H), 5.91 (t, *J* = 6.2 Hz, 1H), 5.79 (dd, *J* = 10.9, 1.5 Hz, 1H), 5.24 (dd, *J* = 2.5, 1.7 Hz, 1H), 4.13 (d, *J* = 10.1 Hz, 1H), 3.98 (dd, *J* = 10.6, 5.5 Hz, 1H), 3.89 (q, *J* = 6.7 Hz, 1H), 3.83 (d, *J* = 10.3 Hz, 1H), 3.69–3.60 (m, 1H), 3.44 (dd, *J* = 6.4, 3.7 Hz, 1H), 3.22–3.04 (m, 2H), 2.89 (td, *J* = 11.0, 3.8 Hz, 1H), 2.77–2.67 (m, 1H), 2.56 (d, *J* = 8.9 Hz, 1H), 2.00–0.50 (m, 71H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.6, 177.9, 156.0, 127.6, 123.1, 104.7, 99.1, 87.9, 75.9, 75.5, 75.1, 73.6, 71.6, 71.1, 68.3, 67.4, 56.1, 49.9, 48.3, 41.1, 41.0, 38.7, 36.3, 36.2, 32.8, 32.6, 31.8, 30.6, 29.9, 29.7, 29.3, 29.2, 29.0, 28.0, 26.8, 26.5, 26.4, 22.6, 22.5, 20.4, 17.9, 16.3, 15.8, 14.12, 14.06, 13.4, 13.0, 12.0, 11.2, 7.0, 6.4 ppm; FT-IR (KBr tablet):

3459 (br, m), 2959 (s), 2930 (s), 2875 (m), 1711 (s), 1534 (m), 1466 (m), 1384 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{51}H_{87}NNaO_{21}^+$ 928.6; Found 929.

C20 tert-butyl carbamate of C20-epi-salinomycin ***16****:* Yield: 45 mg, 49%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 6.34 (d, *J* = 10.7 Hz, 1H), 6.24 (dd, *J* = 10.6, 5.7 Hz, 1H), 4.94 (s, 1H), 4.44 (s, 1H), 4.33 (d, *J* = 6.8 Hz, 1H), 4.20 (d, *J* = 10.1 Hz, 1H), 3.89 (dd, *J* = 11.1, 4.3 Hz, 1H), 3.68 (d, *J* = 10.2 Hz, 1H), 3.57 (d, *J* = 10.2 Hz, 1H), 3.35 (d, *J* = 11.0 Hz, 1H), 2.82 (td, *J* = 10.7, 2.8 Hz, 1H), 2.73–2.56 (m, 2H), 2.20–0.50 (m, 61H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.9, 184.2, 153.6, 127.1, 124.5, 106.8, 99.0, 89.4, 76.5, 75.9, 75.8, 75.0, 74.2, 71.4, 69.9, 67.2, 65.1, 55.3, 51.1, 50.4, 50.3, 40.0, 39.0, 35.9, 32.7, 32.4, 32.2, 28.9, 28.8, 28.0, 27.8, 26.9, 23.8, 20.5, 20.0, 17.5, 16.5, 16.0, 14.5, 13.2, 12.4, 11.8, 10.6, 6.7, 6.4 ppm; FT-IR (KBr tablet): 3295 (br, m), 2959 (s), 2930 (s), 2875 (m), 1730 (s), 1714 (s), 1672 (w), 1571 (s), 1534 (m), 1460 (s), 1408 (m) cm^{-1}; ESI MS (*m/z*): $[M+H]^+$ Calcd for $C_{47}H_{75}NNaO_{12}^+$ 872.5; Found 873.

C20 phenyl carbamate of C20-epi-salinomycin ***17****:* Yield: 58 mg, 23%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^1H NMR (401 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.0 Hz, 2H), 7.05 (dd, *J* = 9.1, 5.4 Hz, 1H), 6.45 (s, 1H), 6.39 (d, *J* = 10.6 Hz, 1H), 6.25 (dd, *J* = 10.5, 5.7 Hz, 1H), 4.99 (d, *J* = 5.7 Hz, 1H), 4.13 (d, *J* = 10.1 Hz, 1H), 3.97 (dd, *J* = 10.8, 5.5 Hz, 1H), 3.90 (d, *J* = 10.4 Hz, 1H), 3.84 (q, *J* = 6.6 Hz, 1H), 3.65 (dd, *J* = 14.2, 6.5 Hz, 2H), 2.89 (td, *J* = 10.8, 3.5 Hz, 1H), 2.74 (dd, *J* = 9.9, 7.2 Hz, 1H), 2.65–2.58 (m, 1H), 2.40–0.50 (m, 56H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.0, 177.8, 152.7, 137.6, 129.0, 128.2, 123.8, 123.5, 118.8, 106.0, 99.0, 90.0, 76.4, 75.3, 74.9, 73.7, 71.5, 71.2, 68.3, 67.3, 56.0, 49.8, 49.0, 40.1, 39.1, 36.6, 36.4, 32.7, 31.4, 29.8, 29.6, 28.0, 26.3, 25.6, 22.8, 22.1, 19.9, 17.9, 16.8, 16.5, 14.2, 13.2, 12.9, 12.0, 11.0, 6.7, 6.4 ppm;

FT-IR (KBr tablet): 3441 (br, m), 3360 (br, m), 2968 (s), 2933 (s), 2875 (m), 1714 (s), 1600 (m), 1538 (m), 1527 (m), 1503 (m), 1457 (m), 1437 (m), 1381 (m) cm^{-1}; ESI MS (m/z): $[M+Na]^+$ Calcd for $C_{49}H_{75}NNaO_{12}^+$ 892.5; Found 893.

*C20 allophanate of C20-epi-salinomycin **18**:* Yield: 48 mg, 39%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^1H NMR (400 MHz, CD_2Cl_2) δ 8.48 (t, *J* = 5.2 Hz, 1H), 6.42 (d, *J* = 10.7 Hz, 1H), 6.15 (dd, *J* = 10.6, 5.7 Hz, 1H), 5.20 (s, *J* = 53.8 Hz, 2H), 4.99 (d, *J* = 5.7 Hz, 1H), 4.28 (dd, *J* = 13.1, 6.2 Hz, 1H), 4.09 (d, *J* = 10.9 Hz, 1H), 3.77 (dd, *J* = 10.9, 4.6 Hz, 1H), 3.73–3.58 (m, 3H), 3.40 (dd, *J* = 12.0, 2.1 Hz, 1H), 3.26 (qd, *J* = 7.2, 5.5 Hz, 2H), 2.82–2.61 (m, 3H), 2.15–0.50 (m, 61H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 218.4, 184.2, 155.8, 154.1, 128.9, 123.3, 106.5, 99.4, 90.2, 76.5, 76.1, 75.6, 74.6, 71.6, 70.0, 68.3, 67.7, 55.9, 51.4, 50.5, 40.4, 39.1, 37.0, 36.3, 35.8, 33.0, 32.8, 32.7, 30.1, 29.4, 28.4, 28.0, 27.2, 24.1, 21.2, 20.3, 17.6, 16.4, 16.2, 15.1, 14.9, 14.6, 13.2, 12.6, 12.3, 10.8, 6.8, 6.6 ppm; FT-IR (KBr tablet): 3494 (br, m), 3348 (br, m), 2968 (s), 2936 (s), 2877 (m), 1720 (s), 1679 (m), 1562 (m), 1539 (m), 1457 (m), 1387 (s) cm^{-1}; ESI MS (*m/z*): $[M+H]^+$ Calcd for $C_{48}H_{80}N_2NaO_{13}^+$ 915.56; Found 915.68.

*4.2.7. General procedure for preparation of C1 propargyl amides of C20 acetate or C20 ethyl carbonate of C20-epi-salinomycin (analogues **19** and **23**)*

To a stirred solution of **7** or **12** (1.0 equiv.) in anhydrous CH_2Cl_2 at 0 °C, the following reagents were added: DCC (1.2 equiv.), HOBt (0.5 equiv., dissolved in a few mL of THF) and propargylamine (2.5 equiv.). The reaction mixtures were warmed to room temperature, stirred for 48 h, and then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-hexane) gave the pure products **19** (44% yield) and **23** (42% yield) as clear

oils. After thrice evaporation to dryness with *n*-pentane, the oily products were completely converted into white amorphous solids. The ^{1}H and ^{13}C NMR spectra of C1/C20 doubly modified analogues **19** and **23** can be found in the Supplementary material (Figures S46–S47 and Figures S58–S59, respectively).

*C1 propargyl amide of C20 acetate of C20-epi-salinomycin **19**:* Yield: 50 mg, 44%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.91 (t, *J* = 5.5 Hz, 1H), 6.40 (d, *J* = 10.6 Hz, 1H), 6.19 (dd, *J* = 10.5, 5.5 Hz, 1H), 4.90 (d, *J* = 5.5 Hz, 1H), 4.41 (ddd, *J* = 17.5, 5.9, 2.5 Hz, 1H), 4.19 (ddd, *J* = 17.6, 4.9, 2.5 Hz, 1H), 4.11 (d, *J* = 9.6 Hz, 1H), 4.02–3.92 (m, 1H), 3.76 (q, *J* = 6.5 Hz, 1H), 3.68 (d, *J* = 9.9 Hz, 2H), 3.42 (dd, *J* = 9.1, 4.8 Hz, 1H), 2.95 (dq, *J* = 14.8, 7.4 Hz, 1H), 2.78 (s, 1H), 2.67 (tt, *J* = 12.7, 6.3 Hz, 1H), 2.64–2.56 (m, 1H), 2.31–2.24 (m, 1H), 2.10 (t, *J* = 2.5 Hz, 1H), 2.00–0.50 (m, 57H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.1, 174.8, 170.2, 127.0, 125.3, 106.0, 98.4, 89.2, 81.3, 79.8, 76.7, 75.1, 73.6, 71.1, 70.9, 69.9, 69.1, 67.2, 54.5, 48.1, 46.9, 39.6, 38.8, 36.7, 36.2, 32.7, 31.5, 30.4, 29.4, 28.8, 28.3, 26.6, 25.1, 22.2, 21.6, 20.9, 20.4, 18.2, 17.3, 16.2, 14.4, 14.3, 13.9, 11.8, 11.5, 8.0, 6.4 ppm; FT-IR (KBr tablet): 3503 (m), 3447 (br, m), 3348 (m), 3312 (m), 3287 (m), 2968 (s), 2936 (s), 2875 (m), 2234 (m), 1744 (s), 1709 (m), 1659 (s), 1527 (m), 1463 (s), 1378 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{47}H_{75}NNaO_{11}^+$ 852.5; Found 853.

*C1 propargyl amide of C20 ethyl carbonate of C20-epi-salinomycin **23**:* Yield: 47 mg, 42%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (403 MHz, CD_2Cl_2) δ 7.01 (t, *J* = 5.2 Hz, 1H), 6.46 (d, *J* = 10.6 Hz, 1H), 6.20 (dd, *J* = 10.5, 5.3 Hz, 1H), 4.81 (d, *J* = 5.3 Hz, 1H), 4.34 (ddd, *J* = 17.5, 5.9, 2.4 Hz, 1H), 4.23–4.10 (m, 4H), 4.05 (d, *J* = 9.4 Hz, 1H), 3.90 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.80–3.62 (m,

4H), 3.46–3.37 (m, 1H), 2.95–2.78 (m, 3H), 2.69 (td, *J* = 10.6, 3.9 Hz, 1H), 2.58 (dt, *J* = 8.9, 2.3 Hz, 1H), 2.15 (t, *J* = 2.4 Hz, 1H), 2.14–0.50 (m, 55H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 213.7, 175.4, 155.2, 128.2, 125.7, 106.8, 99.0, 89.9, 82.2, 80.1, 77.6, 75.8, 74.2, 71.7, 71.3, 70.6, 70.0, 69.7, 64.8, 54.5, 48.5, 47.0, 40.2, 39.2, 37.0, 36.8, 33.2, 32.2, 31.1, 30.0, 29.2, 28.9, 27.2, 25.6, 22.8, 22.2, 20.9, 18.1, 17.6, 16.6, 14.9, 14.8, 14.6, 14.4, 12.1, 11.7, 8.4, 6.9 ppm; FT-IR (KBr tablet): 3479 (br, m), 3353 (br, m), 3313 (m), 2965 (s), 2935 (s), 2877 (s), 2122 (w), 1744 (s), 1712 (s), 1659 (s), 1528 (s), 1460 (s), 1373 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{48}H_{77}NNaO_{12}^+$ 882.5; Found 883.

*4.2.8. General procedure for preparation of C1 propargyl esters of C20 acetate or C20 ethyl carbonate of C20-epi-salinomycin (analogues **20** and **24**)*

To a stirred solution of **7** or **12** (1.0 equiv.) in anhydrous toluene, DBU (1.2 equiv.) and propargyl bromide (~80% in toluene) (3.0 equiv.) were added and the solutions were heated at 90 °C for 6 h. Each solution was then concentrated *in vacuo*. Purification on silica gel using the CombiFlash system (0→50% EtOAc/*n*-hexane) gave the pure products **20** (57% yield) and **24** (35% yield) as clear oils. After thrice evaporation to dryness with *n*-pentane, the oily products were completely converted into white amorphous solids. The ^{1}H and ^{13}C NMR spectra of C1/C20 doubly modified analogues **20** and **24** can be found in the Supplementary material (Figures S49–S50 and Figures S61–S62, respectively).

*C1 propargyl ester of C20 acetate of C20-epi-salinomycin **20**:* Yield: 66 mg, 57%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (401 MHz, $CDCl_3$) δ 6.38 (d, *J* = 10.6 Hz, 1H), 6.21 (dd, *J* = 10.5, 5.7 Hz, 1H), 5.02 (dd, *J* = 15.9, 2.4 Hz, 1H), 4.87 (dt, *J* = 14.3, 3.3 Hz, 1H), 4.06 (dd, *J* = 10.9, 5.7 Hz, 1H),

3.97 (dd, *J* = 9.8, 4.9 Hz, 1H), 3.79–3.71 (m, 1H), 3.64 (dd, *J* = 9.7, 1.5 Hz, 1H), 3.58 (dd, *J* = 10.5, 2.2 Hz, 1H), 3.45–3.40 (m, 1H), 3.15 (dq, *J* = 14.5, 7.3 Hz, 1H), 3.05 (td, *J* = 10.9, 4.3 Hz, 1H), 2.76–2.68 (m, 2H), 2.59 (s, 1H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.20–0.50 (m, 58H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.8, 174.5, 170.2, 127.5, 124.4, 105.7, 98.6, 88.4, 80.2, 78.2, 76.8, 74.7, 73.4, 71.8, 70.9, 69.2, 67.5, 57.1, 52.5, 48.3, 48.1, 39.6, 39.3, 36.5, 36.4, 33.1, 31.8, 30.4, 29.3, 28.3, 26.2, 24.5, 22.7, 21.6, 20.9, 20.5, 19.7, 17.5, 16.2, 14.3, 13.9, 13.0, 11.6, 11.0, 7.3, 6.4 ppm, one signal overlapped; FT-IR (KBr tablet): 3541 (br, m), 3313 (br, m), 3254 (br, m), 2968 (s), 2939 (s), 2875 (m), 2125 (w), 1732 (s), 1626 (w), 1463 (s), 1378 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{47}H_{74}NaO_{12}^+$ 853.5; Found 854.

C1 propargyl ester of C20 ethyl carbonate of C20-epi-salinomycin ***24****:* Yield: 58 mg, 35%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. Strains green with PMA; ^{1}H NMR (403 MHz, CD_2Cl_2) δ 6.39 (d, *J* = 10.6 Hz, 1H), 6.20 (dd, *J* = 10.5, 5.5 Hz, 1H), 5.31 (s, 1H), 4.91 (t, *J* = 2.9 Hz, 2H), 4.76 (d, *J* = 5.5 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.98 (dd, *J* = 11.0, 5.9 Hz, 1H), 3.90 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.72 (q, *J* = 6.9 Hz, 1H), 3.64–3.55 (m, 2H), 3.42 (dd, *J* = 10.7, 2.8 Hz, 1H), 3.10–2.98 (m, 2H), 2.66 (dt, *J* = 10.1, 2.6 Hz, 1H), 2.54 (dd, *J* = 11.2, 8.8 Hz, 3H), 2.15–2.02 (m, 2H), 2.00–0.50 (m, 54H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 214.2, 174.9, 155.0, 128.4, 124.5, 106.1, 99.0, 88.8, 80.0, 78.7, 77.3, 75.0, 74.9, 73.6, 72.2, 71.1, 70.6, 69.6, 64.4, 56.9, 52.7, 48.8, 48.1, 40.0, 39.5, 36.7, 36.6, 33.4, 32.3, 30.9, 29.7, 28.5, 26.6, 24.8, 23.2, 21.9, 20.1, 20.0, 17.6, 16.5, 14.5, 14.4, 14.1, 13.3, 11.8, 11.1, 7.5, 6.6 ppm; FT-IR (KBr tablet): 3545 (br, m), 3458 (br, m), 3311 (m), 2965 (s), 2939 (s), 2877 (s), 2129 (w), 1743 (s), 1713 (s), 1630 (w), 1461 (s), 1374 (s) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{48}H_{76}NaO_{13}^+$ 883.5; Found 883.

*4.2.9. General procedure for preparation of C1 esters of C20 acetate of C20-epi-salinomycin with hydroxamic acids (analogues **21** and **22**)*

To a stirred solution of **7** (1.0 equiv.) in anhydrous CH_2Cl_2 at ambient temperature, DCC (2.0 equiv.) and DMAP (2.0 equiv.) were introduced, and then the respective hydroxamic acid (5.0 equiv.) was added in one portion. The resulting solutions were stirred for 48 h, diluted with CH_2Cl_2 and then washed with saturated NH_4Cl. The organic layers of the reaction mixtures were separated and concentrated *in vacuo* to give clear oils. Purification on silica gel using the CombiFlash system (0→30% EtOAc/*n*-hexane) gave the pure products **21** and **22** (17–27% yield) as clear oils. After thrice evaporation to dryness with *n*-pentane, the oily products were completely converted into white amorphous solids. The ^{1}H and ^{13}C NMR spectra of esters **21** and **22** can be found in the Supplementary material (Figures S52–S53 and Figures S55–S56, respectively).

*C1 benzhydroxamic acid ester of C20 acetate of C20-epi-salinomycin **21**:* Yield: 31 mg, 27%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 11.04 (s, *J* = 12.0 Hz, 1H), 8.21–8.09 (m, 2H), 7.57–7.49 (m, 1H), 7.49–7.38 (m, 2H), 6.40 (d, *J* = 10.6 Hz, 1H), 6.16 (dd, *J* = 10.5, 5.5 Hz, 1H), 4.80 (d, *J* = 5.5 Hz, 1H), 4.03 (ddd, *J* = 23.0, 12.0, 7.2 Hz, 2H), 3.83 (dd, *J* = 10.1, 2.4 Hz, 1H), 3.77–3.68 (m, 2H), 3.48 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.21–3.11 (m, 1H), 2.95 (dt, *J* = 17.3, 7.4 Hz, 1H), 2.64 (s, 1H), 2.56 (dd, *J* = 7.2, 2.2 Hz, 1H), 2.20–2.14 (m, 1H), 2.20–0.50 (m, 57H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 216.0, 173.9, 170.3, 165.4, 132.3, 131.5, 128.6, 128.3, 126.8, 126.0, 106.2, 98.8, 90.1, 80.1, 77.0, 75.0, 73.4, 72.3, 71.1, 68.9, 67.4, 53.9, 46.6, 46.4, 40.1, 38.7, 37.2, 36.7, 32.8, 31.1, 31.0, 29.9, 28.6, 26.8, 25.8, 23.6, 22.2, 21.0, 20.1, 17.2, 17.0, 16.3, 14.9, 14.7, 12.0, 11.0, 7.9, 6.7 ppm, one signal overlapped; FT-IR (KBr tablet): 3509 (br, m), 3313 (br, m), 2959 (s), 2936 (s), 2880 (m), 1785 (s), 1741 (m), 1703 (s), 1697 (m), 1604

(w), 1583 (w), 1504 (w), 1463 (m), 1373 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{51}H_{77}NNaO_{13}^+$ 934.5; Found 934.

C1 salicylhydroxamic acid ester of C20 acetate of C20-epi-salinomycin ***22****:* Yield: 35 mg, 17%. Isolated as a white amorphous solid, >95% pure by NMR and a single spot by TLC. UV-active and strains green with PMA; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 11.74 (s, 1H), 11.20 (s, 1H), 8.33 (ddd, *J* = 9.6, 8.1, 1.4 Hz, 1H), 7.41 (ddd, *J* = 8.7, 7.3, 1.5 Hz, 1H), 6.96–6.92 (m, 1H), 6.85 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 1H), 6.42 (dd, *J* = 10.6, 2.4 Hz, 1H), 6.17 (ddd, *J* = 10.5, 5.4, 2.9 Hz, 1H), 4.80 (d, *J* = 5.5 Hz, 1H), 4.07–3.96 (m, 2H), 3.88 (dd, *J* = 10.1, 2.5 Hz, 1H), 3.77–3.67 (m, 2H), 3.49 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.36 (t, *J* = 4.6 Hz, 1H), 3.17 (ddd, *J* = 15.2, 10.5, 5.9 Hz, 1H), 3.01–2.91 (m, 1H), 2.62–2.55 (m, 1H), 2.30–0.50 (m, 58H) ppm; ^{13}C NMR (101 MHz, CD_2Cl_2) δ 216.5, 173.6, 170.2, 168.6, 161.9, 134.9, 127.8, 126.8, 126.3, 126.1, 118.9, 118.1, 112.0, 106.2, 98.8, 90.2, 80.0, 76.9, 75.1, 73.4, 72.2, 71.1, 68.8, 67.4, 46.5, 46.2, 40.1, 38.6, 37.2, 36.8, 32.8, 31.0, 30.9, 29.9, 28.5, 26.7, 26.1, 23.5, 22.3, 21.0, 20.0, 17.2, 16.7, 16.3, 15.0, 14.8, 14.6, 11.9, 11.0, 8.0, 6.7 ppm; FT-IR (KBr tablet): 3517 (br, m), 3301 (br, m), 2963 (s), 2933 (s), 2875 (m), 1787 (s), 1744 (s), 1703 (s), 1650 (s), 1609 (m), 1565 (w), 1510 (m), 1481 (m), 1457 (m), 1367 (m) cm^{-1}; ESI MS (*m/z*): $[M+Na]^+$ Calcd for $C_{51}H_{77}NNaO_{14}^+$ 950.5; Found 950.

4.3. In vitro biological studies

The protocols for the cultivation of bloodstream form of *T. brucei* 427-221a [30] and human myeloid HL-60 cells [31], the screening assay, and the swelling experiments can be found either in the Supplementary material or in the reference literature [9,18,26–27].

Supporting Information

Additional figures presenting the NMR and ESI MS spectra of the newly synthesized derivatives of salinomycin are freely available *via* the Internet at http://xxx.

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