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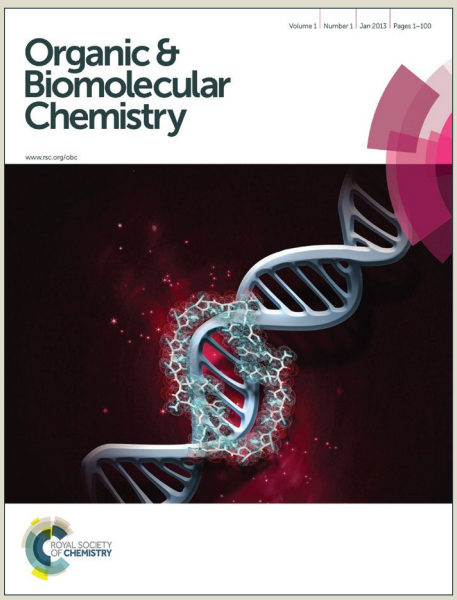

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ARTICLE

Structure–activity & structure–toxicity relationship study of salinomycin diastereoisomers and their benzoylated derivatives

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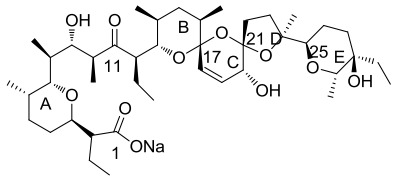
Salinomycin diastereoisomers and their benzoylated derivatives were synthesized and evaluated with both antiproliferative activities and neurotoxicity *in vitro*. The results indicated that the stereoscopic configurations of the spiro C17 and C21 atoms as well as the benzoyl groups of *O*-20 on the rigid B/C/D spiro-ketal structures are crucial for the biological activities and neural toxicity. In general, there are some positive correlations between the antiproliferative activities and neurotoxicity in these salinomycin derivatives, indicating possibly similar mechanisms of the actions.

Key words Salinomycin Diastereoisomers, Structure–Activity Relationship, Structure–Toxicity Relationship

Introduction

Salinomycin sodium salt (**1, Fig1**) is a kind of polyether antibiotics isolated from *Streptomyces albus*. Up to now, it has been used in broiler batteries and other poultry as an anticoccidial drug and also fed to ruminants and pigs to improve nutrient absorption and feed efficiency.[1] A study in 2009 found salinomycin kills breast cancer stem cells (CSCs) *in vitro* and inhibits mammary tumor growth in mice. Its effect is 100 times higher than the clinically used antitumor drug taxol[2]. Follow-up publications demonstrated that salinomycin also targets other CSCs as well as differentiated cancer cells that display efficient mechanisms of resistance to cytotoxic drugs and radiation in different types of human cancers, including leukemia, breast cancer, gastric cancer, lung adenocarcinoma, osteosarcoma, colorectal cancer, squamous cell carcinoma, prostate cancer and so on[3]. Several possible mechanisms of salinomycin were illuminated, such as induction of apoptosis and cell death, interference with ABC transporters, inhibition of oxidative phosphorylation and inhibition of the Wnt/β-catenin signaling pathway, but the exact mechanisms were still not fully elucidated[4].



salinomycin sodium salt(1)

Figure 1 The structure of salinomycin

The encouraging biological activities of salinomycin have drawn attentions of many medicinal chemists. Huczynski's group synthesized several C1-amides, C1-esters as well as C1-conjugated derivatives of salinomycin, some of which showed slightly stronger antitumor activities[5]. Daniel Strand's group synthesized several C20 hydroxyl acylated salinomycin analogs, which displayed IC_{50} values down to one fifth that of the native structure against breast cancer cells[6]. Their followed study found C20-deoxy-saliomycin reduces significant anti-cancer activity, which emphasizes the importance of substitution at C20 for the activity.[7]

The 6-6-5 spiro-ketal structure (C-ring) in salinomycin is relatively infrequent in natural products[8], which forms a relatively fixed conformation in the molecule. However, how the spatial configurations of the stereogenic centers on C-ring affect antiproliferative activities is still unclear.

In addition, one important caveat for the potential clinical application of salinomycin is its considerable neural toxicity. Boehmerle and colleagues have showed that salinomycin in concentrations effective against CSCs exerts profound toxicity towards both dorsal root ganglia as well as Schwann cells[9]. This toxic effect is mediated by elevated cytosolic Na^+ concentrations by salinomycin, which in turn causes an increase of cytosolic Ca^{2+} by means of Na^+/Ca^{2+} exchangers (NCXs) in the plasma membrane and the mitochondria. Elevated Ca^{2+} then leads to calpain activation, which triggers caspase-dependent apoptosis involving caspases 12, 9 and 3. Recently they found that inhibition of the mitochondrial Na^+/Ca^{2+} exchanger partially prevents the development of salinomycin-induced

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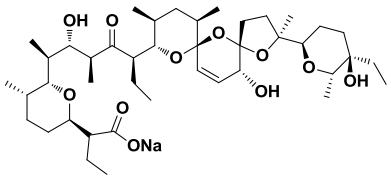
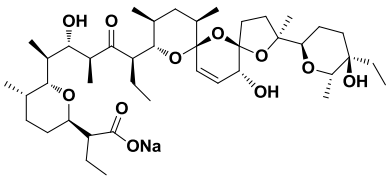
neuropathy *in vivo*, but does not reduce salinomycin's antineoplastic efficacy[10]. These studies inspired us that direct inhibition of sodium ion transport capacity of salinomycin may reduce its toxicity.

Paulus and colleagues analyzed the solid-state and solution structures of the salinomycin-sodium complexes. They found that different environments tend to stabilize different conformations of the outer sphere, while the complexation pattern and the geometry of the coordination sphere of the sodium ion remain unaffected[11]. Four oxygens including 1a-O, 11-O, 21-O and 25-O are coordinating the central sodium atom, which form a relatively fixed conformation. We supposed that breaking the combination pattern through inversions of the configurations of spiro C17 and C21 atoms on the rigid spiro-ketal structure (B/C/D rings) may possibly affect the conformations of salinomycin and then decrease neurotoxicity by inhibition of the combination of the sodium ions[12].

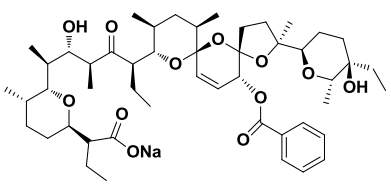
For these purposes, salinomycin diastereoisomers such as 17-*epi*-salinomycin (**2**) and 17, 21-di-*epi*-salinomycin (**3**)[13]as well as their 20-*O*-benzoylated derivatives (**4-5**) were synthesized and evaluated of both pharmacological activities and toxicity to help us understand the relationships of the structures and functions (**Fig 2**).



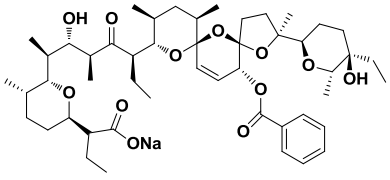
17-*epi*-salinomycin sodium salt(2)



17,21-di-*epi*-salinomycin sodium salt(3)



17-*epi*-20-*O*-Bz-salinomycin sodium salt(4)



17,21-di-*epi*-20-*O*-Bz-salinomycin sodium salt(5)

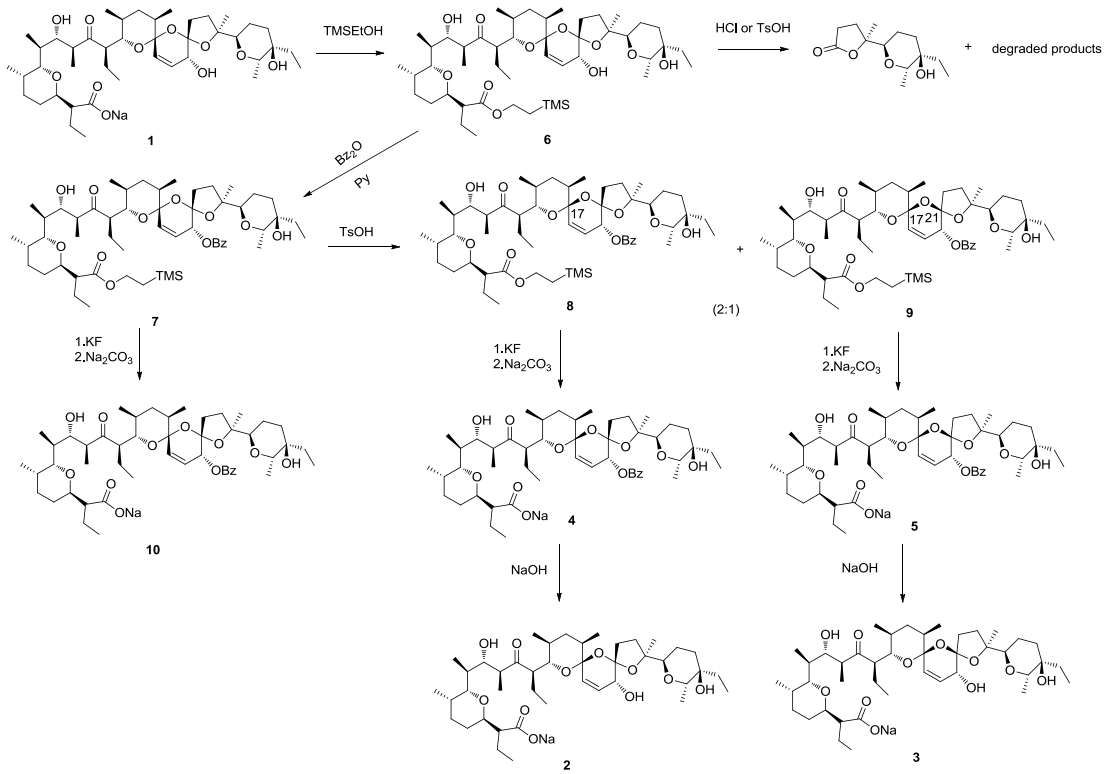
Figure 2 The structures of salinomycin diastereoisomers and their benzoylated derivatives (sodium salt)

Results and discussion

Kocienski and co-workers pointed out that 17-epi-salinomycin is more stable than salinomycin, because it possesses three stabilising anomeric interactions but does not suffer from the unfavourable 1, 3-dipole-dipole interaction exhibited by salinomycin[13]. Thus treatment of 20-*O*-acetylated salinomycin with camphorsulfonic acid (Kocienski and co-workers have not given the details) [13] or *p*-toluenesulfonic acid (0.01M in CH_2Cl_2) resulted in complete isomerisation to the 17-epimer. However, salinomycin can't tolerate the similar acid condition such as *p*-toluenesulfonic acid (0.01M in CH_2Cl_2), which leads to degradation from spiro ketal fragment immediately[14]. These results indicated that the acyl group could increase the stability of the substrate to acid by the possible electron-withdrawing effect and spatial effect. Daniel Strand's group have developed a protection strategy of carboxyl with TMSEt-ester for the synthesis of the acylated salinomycin derivatives (**Scheme 1**) [6]. When 20-benzoylated salinomycin-TMSEt-ester **7** was treated with *p*-toluenesulfonic (0.3M in CH_2Cl_2) within 5 minutes, the 17-*epi*-product **8** and 17,21-di-*epi*-product **9** were afforded in 50% and 25% yield respectively. Prolonging reaction time also led to degradation of products. The structure of compound **9** was confirmed by X-ray crystallography (CCDC 1434828), which adopted a compact conformation as a Y shape (**Fig 3**). Hydrolysis of TMSEt-ester groups in compounds **7, 8** and **9** with KF gave their 20-*O*-benzoylated salts **10**, **4** and **5** respectively. Treatment of compound **10** with *p*-toluenesulfonic acid (0.01M in CH_2Cl_2) could also afford its 17-epimer **4**. Hydrolysis of the benzoyl groups in compounds **4** and **5** then afforded 17-*epi*-salinomycin sodium salt (**2**) and 17, 21-di-*epi*-salinomycin sodium salt (**3**) respectively (**Scheme 1**).

The antiproliferative activities of salinomycin diastereoisomers and their benzoylated derivatives were evaluated in HT-29 colorectal cancer[15], HGC-27 gastric cancer and triple negative MDA-MB-231 human breast cancer cells[16]using MTT assay (**Table 1**). Salinomycin salt showed moderate inhibitory activities in above three cancer cell lines with IC_{50} values between 3-10 μM, while its 17-epimer (**2**) reduced significantly activities (inhibition rate <10% at 10μM). In contrast, 17, 21-di-*epi*-salinomycin (**3**) showed better antiproliferative activities in all of the cancer cells, which was more potent than salinomycin. The above results indicated that the relative orientations of the two oxygen atoms in B and D rings are crucial: when they are both under the C-ring in the natural product or above the C-ring in 17, 21-di-*epi* salinomycin respectively, the molecules adopt relatively more compact conformations and show better biological activities. In contrast, when the two oxygen atoms are on opposite side of the C-ring in 17-epimer, the molecule adopts relatively more open conformation and the antiproliferative activities are declined. The 20-*O*-benzoylated derivatives exhibited more significant differences in biological activities. 20-*O*-benzoylated

salinomycin (**10**) showed the best activities, which was 100 times more potent than the natural product. However, 20-*O*-benzoylated 17, 21-di-*epi*-salinomycin **5** was 4-7 fold less potent than the hydroxyl free product **3**.



Scheme 1 Synthesis of salinomycin diastereoisomers and their benzoylated derivatives (sodium salts)

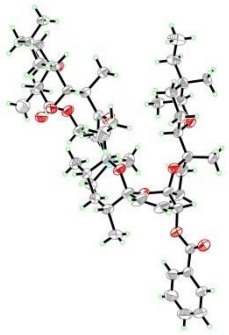


Figure 3 The X-ray crystal structures of 9

Then the neurotoxicitis of the compounds were evaluated with neurons in cerebral cortex of E18 rats.[17] Salinomycin showed worth noting neurotoxicity with similar IC_{50} value as its antiproliferative activities. As expected, 17-epimer (**2**) and its benzoylated derivative (**4**) exhibited reduced neurotoxicity by the change of the molecule conformations. To our surprise, 17, 21-*di*-diastereoisomer (**3**), with almost the opposite conformation to salinomycin, also showed comparative neurotoxicity with salinomycin. It may suggest that the sodium ion could also be combined in this compact conformation. Regrettably, 20-*O*-Bz-salinomycin (**10**) showed almost 100 times more poisonous than the natural product, indicating the benzoyl group in the natural product is more beneficial to sodium ion transport[18]. In contrast, the benzoyl groups have some positive effects on its diastereoisomers **4** and **5**, leading to the reduction of the neurotoxicity.

Table 1 Antiproliferative activities and neurotoxicity of the compounds *in vitro* (MTT assay, IC_{50} [μM])

No	HT-29	HGC-27	MDA-MB-231	Neuron cell
1	3.209±0.343	8.180±0.104	>10.0	5.49
2	>10.0	>10.0	>10.0	9.50
3	0.754±0.048	0.615±0.184	5.695 ±1.439	4.01
4	>10.0	>10.0	>10.0	>10.0
5	2.803±0.094	4.135±1.338	6.564 ±0.713	>10.0
10	0.024±0.001	0.026±0.002	0.038 ±0.008	0.076

Conclusion

The stereogenic centers on the rigid B/C/D spiro-ketal structure in salinomycin play important roles in biological activities and neurotoxicity by affecting the molecular conformations. More ever, the benzoyl groups of 20-hydroxyls have significantly different influences on salinomycin and its diastereoisomers. In general, there are some positive correlations between the biological activities and neurotoxicity. The compounds with compact conformations showed better biological activities and more serious neurotoxicity, while the diastereoisomers with open conformations almost lost their pharmacological activities, suggesting possibly similar mechanisms of the actions.[19,20]

Experimental

1.Chemistry

1.1 General experimental information

All reactions were performed in glassware containing a Tefloncoated stir bar. Solvents and chemical reagents were obtained from commercial sources and used without further purifications. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz, and the data were recorded using $CDCl_3$ as the solvent. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard. High-resolution mass spectra were obtained in the ESI mode. Flash column chromatography on silica gel (200-300 mesh) was used for the routine purification of reaction products. The column output was monitored by TLC on silica gel (100-200 mesh) precoated on glass plates(15×50 mm), and spots were visualized by 5% vanillin sulfuric acid/ethanol solution.

1.2 Preparation of 20-O-Bz-salinomycin-EtTMS-ester (7)[13]

To a solution of salinomycin-EtTMS-ester **6** (2.0g, 2.35mmol) in pyridine (3 mL) was added benzoic anhydride (2.0g, 8.85mmol) and DMAP at r.t. The resulting mixture was stirred at room temperature for 24h and then poured into ice water. The two phases were separated, and the organic layer was redissolved with ethyl acetate (30 mL) and washed with 0.1M HCl(aq) 10mL, 0.1M NaOH(aq) 10 mL and water 10mL, then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified via silica column chromatography with petroleum ether/ethyl acetate (4:1) as eluent to provide compound **7** (2.0g) in 89% yield.

*1.3 Preparation of intermediates **8** and **9***

To a solution of 20-*O*-Bz-salinomycin TMSEt-ester (0.92g, 0.96mmol) in CH_2Cl_2 (3 mL) was added *p*-toluenesulfonic acid monohydrate (180mg, 0.95mmol) at r.t. The resulting mixture was stirred at room temperature within 5 minutes (the color was getting to be yellow) and then neutralized by saturated $NaHCO_3$ (aq). The two phases were separated, and the aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified via silica column chromatography with petroleum ether/ethyl acetate (8:1) as eluent to provide compounds **8** (460mg) in 50% yield and **9** (230mg) in 25% yield.

Compound **8** colorless oil, $[\alpha]_D^{20}$ = -111.3 (*c* = 2.9, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.6 Hz, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 2H), 6.05 (dd, *J* = 9.9, 6.0 Hz, 1H), 5.79 (d, *J* = 9.9 Hz, 1H), 5.30 (d, *J* = 6.0 Hz, 1H), 4.44 (dtd, *J* = 28.5, 11.0, 6.3 Hz, 2H), 4.15 – 3.95 (m, 2H), 3.81 (d, *J* = 10.7 Hz, 1H), 3.69 (dd, *J* = 16.5, 8.5 Hz, 2H), 3.35 (d, *J* = 10.0 Hz, 1H), 3.21 – 2.92 (m, 3H), 2.89 – 2.48 (m, 3H), 2.17 – 2.05 (m, 1H), 2.01 – 0.50 (m, 57H), 0.07 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.7, 175.9, 166.4, 135.4, 132.8, 130.5, 129.9, 128.3, 123.9, 106.3, 99.3, 86.4, 77.7, 75.1, 73.1, 71.7, 71.0, 68.9, 67.1, 63.7, 57.0, 48.8, 48.4, 38.7, 36.4, 36.4, 35.7, 34.1, 33.1, 30.3, 29.3, 28.1, 26.2, 23.7, 22.7, 21.9 , 20.9, 19.7, 18.2, 17.3, 16.6, 14.2, 13.8, 13.3, 11.9, 11.0, 7.2, 6.5, -1.4. HRMS-ESI (m/z): $[M + Na]^+$ Calcd for: $C_{54}H_{86}O_{12}SiNa$, 977.5786.; Found: 977.5747. FTIR (neat): 3527 (s), 2965 (s), 2875 (w), 1714 (s), 1602 (w), 1584 (w), 859(m), 838(m)cm^{-1}.

Compound **9** colorless oil, $[\alpha]_D^{20}$ = -131.2 (*c* = 1.25, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 7.97 (m, 2H), 7.62 – 7.50 (m, 1H), 7.51 – 7.36 (m, 2H), 6.19 (dd, *J* = 10.1, 5.6 Hz, 1H), 5.95 (d, *J* = 10.1 Hz, 1H), 5.19 (d, *J* = 5.6 Hz, 1H), 4.64 – 4.41 (m, 2H), 4.19 – 4.10 (m, 1H), 4.05 (dd, *J* = 11.2, 5.8 Hz, 1H), 3.78 (q, *J* = 6.8 Hz, 1H), 3.68 (dd, *J* = 9.8, 2.0 Hz, 1H), 3.61 (dd, *J* = 10.5, 2.5 Hz, 1H), 3.51 (dd, *J* = 10.7, 3.5 Hz, 1H), 3.18 (dd, *J* = 10.2, 7.4 Hz, 1H), 3.02 (td, *J* = 11.0, 4.5 Hz, 2H), 2.85 (dt, *J* = 12.3, 2.5 Hz, 1H), 2.35 – 0.54 (m, 57H), 0.08 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.5, 176.1, 165.9, 135.2, 133.2, 130.2, 129.7, 128.5, 122.9, 105.8, 96.6, 87.0, 79.7, 77.3, 74.9, 71.7, 71.0, 68.5, 63.8, 58.1, 53.5, 49.8, 48.9, 37.8, 36.5, 35.5, 34.9, 32.4, 31.0, 30.6, 28.9, 28.0, 26.2, 23.5, 22.7, 21.0, 19.8, 19.7, 18.8, 17.4, 16.8, 14.3, 13.8, 13.0, 11.9, 10.9, 7.3, 6.4, -1.5. HRMS-ESI (m/z): $[M + Na]^+$ Calcd for: $C_{54}H_{86}O_{12}SiNa$, 977.5786.; Found: 977.5782. FTIR (neat): 3535 (s), 2964 (s), 2876 (w), 1715 (s), 1603 (w), 1584 (w), 860(m), 839(m)cm^{-1}.

*1.4 Preparation of 17-epi-20-O-Bz-salinomycin sodium salt **(4)**, 17,21-di-epi-20-O-Bz-salinomycin sodium salt **(5)** and 20-O-Bz-salinomycin sodium salt **(10)***

To a solution of compound **7**, **8** or **9** in DMF was added KF (10.0 equiv). The resulting solution was stirred at 80℃ until complete consumption of starting material (typically12 hours, TLC control, petroleum ether/ethyl acetate=2:1), then concentrated under reduced pressure. Purification by flash chromatography with petroleum ether/ethyl acetate (3:1) as eluent provided the product as potassium salt forms. The product mixture was re-dissolved in EtOAc (10 mL) and washed with 0.1 M Na_2CO_3 (3 x 10 mL). The organic layer was separated, dried using a phase separator, and concentrated several times from n-pentane to give sodium salt as foam.

*1.4.1.17-epi-20-O-Bz-salinomycin sodium salt **(4)***

78%,$[\alpha]_D^{20}$ = -122.1 (*c* =3.73, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 2H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 2H), 6.03 (dd, *J* = 10.0, 5.3 Hz, 1H), 5.79 (d, *J* = 10.0 Hz, 1H), 5.36 (d, *J* = 5.3 Hz, 1H), 4.11 (t, *J* = 9.4 Hz, 1H), 4.00 (dd, *J* = 11.0, 5.7 Hz, 1H), 3.80 (d, *J* = 10.5 Hz, 1H), 3.70 (dt, *J* = 23.0, 8.4 Hz, 2H), 3.38 (d, *J* = 10.1 Hz, 1H), 3.05 (t, *J* = 8.8 Hz, 1H), 2.95 – 2.82 (m, 1H), 2.82 – 2.61 (m, 2H), 2.17 – 0.53 (m, 51H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.0, 166.5, 134.5, 132.8, 130.5, 129.9, 128.3, 125.3, 106.6, 98.8, 86.0, 77.1, 76.5, 75.0, 72.9, 71.3, 71.1, 68.9, 67.5, 56.4, 49.3, 49.0, 38.8, 36.3, 36.3, 35.4, 34.5, 32.7, 30.5, 29.7, 29.1, 28.2, 26.4, 23.0, 22.5, 20.7, 20.0, 18.3, 16.5, 14.3, 13.7, 13.3, 11.9, 11.1, 7.2, 6.4. HRMS-ESI (m/z): $[M + H]^+$ Calcd for: $C_{49}H_{74}NaO_{12}$, 877.5078.; Found : 877.5074. FTIR (neat): 3340 (s, brs), 2963 (s), 2876 (w), 1715 (s), 1573 (s) cm^{-1}.

*1.4.2.17,21-di-epi-20-O-Bz-salinomycin sodium salt **(5)***

83%,$[\alpha]_D^{20}$ = -34.7 (*c* = 1.5, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 7.7 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 6.14 (s, 1H), 6.04 (d, *J* = 9.7 Hz, 1H), 5.84 (dd, *J* = 9.7, 3.1 Hz, 1H), 4.57 (d, *J* = 10.9 Hz, 1H), 4.28 (d, *J* = 10.5 Hz, 1H), 4.07 (q, *J* = 6.9 Hz, 1H), 3.95 (dd, *J* = 11.2, 4.7 Hz, 1H), 3.73 (d, *J* = 10.1 Hz, 1H), 3.56 (dd, *J* = 12.2, 3.0 Hz, 1H), 3.04 (dd, *J* = 10.7, 6.5 Hz, 1H), 2.88 (td, *J* = 11.3, 3.8 Hz, 1H), 2.60 (h, *J* = 8.9 Hz, 3H), 2.29 (td, *J* = 12.5, 7.2 Hz, 1H), 2.23 – 0.47 (m, 49H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.5, 182.2, 165.9, 133.2, 132.1, 131.7, 129.9, 128.5, 110.9, 98.2, 88.0, 76.0, 73.0, 71.8, 71.6, 69.9, 68.4, 55.6, 51.1, 45.3, 39.0, 36.6, 36.1, 31.3, 30.8, 30.7, 29.7, 29.6, 28.0, 26.8, 25.9, 24.1, 23.9, 20.4, 20.2, 17.4, 17.3, 16.0, 15.7, 14.1, 13.9, 12.4, 10.8, 6.8, 6.6. HRMS-ESI (m/z): $[M + H]^+$ Calcd for: $C_{49}H_{74}NaO_{12}$, 877.5078.; Found: 877.5086. FTIR (neat): 3309 (s, brs), 2963 (s), 2876 (w), 1709 (s), 1573 (s) cm^{-1}.

*1.4.3.20-O-Bz-salinomycin sodium salt **(10)***[13]

84%, $[\alpha]_D^{20}$ = -26.7 (*c* = 0.4, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.6 Hz, 2H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 2H), 6.24 (dd, *J* = 10.9, 2.7 Hz, 1H), 6.02 (d, *J* = 10.8 Hz, 1H), 5.74 (s, 1H), 4.40 – 4.33 (m, 1H), 4.30 (d, *J* = 10.3 Hz, 1H), 4.11 (q, *J* = 7.2 Hz, 1H), 3.92 – 3.86 (m, 1H), 3.68 (d, *J* = 10.1 Hz, 1H), 3.53 (d, *J* = 10.0 Hz, 1H), 3.36 (d, *J* = 11.9 Hz, 1H), 2.80 (td, *J* = 11.2, 3.0 Hz, 1H), 2.76 – 2.66 (m, 1H), 2.67 – 2.57 (m, 1H), 2.41 – 2.21 (m, 1H), 2.10 – 0.57 (m, 51H).

*1.5Preparation of 17-epi-salinomycin sodium salt **(2)**, 17,21-di-epi-salinomycin sodium salt **(3)***

To a solution of compound **4** or **5** in methanol (1mL) was added NaOH (2.0 equiv) at r.t. The resulting solution was stirred until complete consumption of starting material (typically 1 hour, TLC control, petroleum ether/ethyl acetate=2:1), then diluted with EtOAc (10 mL) and washed with 0.1 M Na_2CO_3 (3 x 10 mL). The organic layer was separated, dried using a phase separator, and concentrated several times from n-pentane to give sodium salt as foam**.**

*1.5.1.17-epi-salinomycin sodium salt **(2)***

67%, $[\alpha]_D^{20}$ = -70.7 (*c* = 1.5, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (dd, *J* = 9.9, 3.1 Hz, 1H), 5.63 (d, *J* = 9.9 Hz, 1H), 4.26 – 4.05 (m, 2H), 3.99 (dd, *J* = 11.0, 5.3 Hz, 1H), 3.80 (q, *J* = 6.8 Hz, 1H), 3.73 (d, *J* = 10.5 Hz, 1H), 3.66 (d, *J* = 9.8 Hz, 1H), 3.56 (d, *J* = 8.4 Hz, 1H), 3.01 (t, *J* = 8.7 Hz, 1H), 2.86 (dq, *J* = 11.2, 6.2, 3.9 Hz, 1H), 2.70 – 2.55 (m, 1H), 2.35 – 2.09 (m, 3H), 2.03 (s, 1H), 1.97 – 0.53 (m, 54H). ^{13}C NMR (100MHz, $CDCl_3$) δ 216.2, 180.6, 133.5, 130.3, 109.4, 97.3, 86.0, 77.3, 77.0, 75.4, 75.2, 73.6, 71.6, 70.8, 67.8, 66.8, 55.3, 49.7, 48.2, 38.8, 36.4, 36.2, 35.8, 32.7, 31.6, 30.7, 29.2, 28.2, 26.5, 25.3, 22.8, 21.8, 20.1, 18.8, 18.0, 16.1, 14.6, 13.4, 12.1, 11.2, 7.4, 6.4. HRMS-ESI (m/z): $[M + H]^+$ Calcd for: $C_{42}H_{70}NaO_{11}$, 773.4816.; Found : 773.4831. FTIR (neat): 3361 (s, brs), 2933 (s), 2876 (w), 1712 (m), 1573 (s) cm^{-1}.

*1.5.2.17, 21-di-epi-salinomycin sodium salt **(3)***

82%, $[\alpha]_D^{20}$ = -3.3 (*c* = 0.9, CH_2Cl_2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.19 (d, *J* = 9.6 Hz, 1H), 5.63 (dd, *J* = 9.6, 3.0 Hz, 1H), 4.38 (d, *J* = 10.8 Hz, 1H), 4.21 (d, *J* = 10.5 Hz, 1H), 4.00 (q, *J* = 7.1 Hz, 1H), 3.79 (dd, *J* = 11.1, 4.8 Hz, 1H), 3.63 (d, *J* = 10.8 Hz, 2H), 3.01 – 2.80 (m, 2H), 2.54 (dd, *J* = 13.1, 5.1 Hz, 1H), 2.48 (d, *J* = 9.3 Hz, 1H), 2.40 – 0.45 (m, 54H).;^{13}C NMR (100 MHz, $CDCl_3$) δ 220.3, 182.3, 136.5, 129.5, 112.9, 98.1, 87.5, 77.2, 75.2, 72.4, 72.1, 71.3, 71.3, 70.1, 66.4, 55.8, 51.3, 45.9, 39.1, 36.7, 35.8, 31.4, 30.8, 29.9, 29.0, 27.9, 26.9, 26.0, 24.2, 24.0 , 20.4 , 20.3 , 17.5 , 16.9 , 16.1 , 15.9, 14.2, 12.6, 10.8, 6.7, 6.5. HRMS-ESI (m/z): $[M + H]^+$ Calcd for: $C_{42}H_{70}NaO_{11}$, 773.4816.; Found : 773.4811. FTIR (neat): 3346 (s, brs), 2927 (s), 2876 (w), 1706 (s), 1571 (s) cm^{-1}.

*1.6 Experiment and Crystal data and structure refinement details of compound **9**:*

A suitable crystal was selected and analyzed on a Xcalibur, Atlas, Gemini ultra diffractometer. The crystal was kept at 293(2) K during data collection. Using Olex2, the structure was solved with the Superflip structure solution program using Charge Flipping and refined with the ShelXL refinement package using Least Squares minimisation.

Crystal Data for $C_{55}H_{88}O_{11}Si$ (*M* =953.34 g/mol): monoclinic, space group $P2_1$ (no. 4), a=12.1784(3) Å b=10.4550(2) Å c=22.4818(4) Å α=90.0000° β=99.8798(18)° γ=90.0000° V=2820.05(10) Å3 Z=2, μ(CuKα) = 0.803 mm^{-1}, *Dcalc* = 1.123 g/cm^3, 41478 reflections measured (7.368° ≤ 2^Θ ≤ 134.002°), 10003 unique (R_{int} = 0.0390, R_{sigma} = 0.0221) which were used in all calculations. The final R_1 was 0.0447 (I > 2σ(I)) and wR_2 was 0.1313 (all data).

2. Biological evaluation

2.1. In vitro cytotoxicity evaluation

Each test solution for *in vitro* assay was prepared by diluting with DMSO (Sigma-Aldrich). Solutions for *in vitro* assay were dissolved by culture medium RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco) to obtain a series of concentrations. All cells used in the research were prepared at 3.5×10^4 cells/ml concentration and each 100 μL cells suspension was seeded in 96-well cell microplates (Corning) for 24 hours (37℃, 5%CO_2). Then each solution was added and incubated for another 48 hours. For the control group, equivalent concentration of DMSO (final concentration 0.1%) was added. MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazoliumbromide) (Sigma-Aldrich) method was employed to measure the number of surviving cells and recorded the OD values at 570 nm using microplate reader (Perkin Elmer). All the experiments were performed in triplicate and the IC_{50} values were calculated using Prism Graphpad software.

2.2. Cell culture of neurons

Cerebral cortex neurons were prepared from SD embryonic rats (E18). Briefly, cerebral cortex were dissected and dissociated with 0.25% trypsin (Gibco) in phosphate-buffered saline (PBS). Dissociated cell suspensions were plated at 1.5×10^5 cells/cm^2 on 96-well cell microplates coated with 0.1% polyethylenimine in Neurobasal (Gibco) supplemented with 1% B27 (Gibco), 0.5 mM glutamine (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). Three days after plating, nonneuronal cells were removed by adding 5 μM cytarabine. Before experiments, cells were cultured for 10 days at 37°C in a 5% CO_2/95% air humidified incubator.

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