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Short communication

Design and synthesis of conformationally constrained salinomycin derivatives

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ABSTRACT

Two conformationally restricted salinomycin derivatives by tethering the hydroxyl groups at C1 and C20 with different chain length were designed and synthesized. The cyclic derivatives showed better biological activities than C1/C20 modified derivatives, indicating the importance of the compact conformation for the ion binding capacity. In addition, the length of the connective chain plays critical role in the biological activities, thus cyclic the derivative **7** preserved some pharmacological activity but derivative **5** with two carbon atom shorter chain showed significantly reduced activity. The conformations of the two cyclic salinomycin derivatives were analyzed by ROESY spectrum in DMSO-d_6, indicating derivative **7** may adopt more appropriate conformations for the coordinate with alkali metal ion than derivative **5**, which has a closer distance between H3 and H25.

1. Introduction

Salinomycin (SAL, 1, Fig. 1) is a kind of polyether isolated from *Streptomyces albus*, which has a wide range of biological activities, such as antibacterial [1], anticoccidial [2] and anti-tumor including anti cancer stem cells [3]. Until now, several groups have synthesized and assayed salinomycin derivatives, such as C1-ester [4], amide [5] and conjugates with other active substances [6], and C9/C20/C28-hydroxyl acylated analogs [7] as well as salinomycin diastereoisomers [8]. According to the structure–activity relationship study, the combination and transport ability of alkali metal ion of salinomycin are proved to be crucial to its biological activities [9]. According to the literature, salinomycin interferes with transmembrane K^+ potential and promotes the efflux of K^+ from mitochondria and cytoplasm, leading to apoptosis in CSCs [3].

The solution structure of the ionophore metal complex salinomycin-Na in $CDCl_3$ was firstly determined by NMR and molecular dynamics calculations by Mronga's group [10]. In a followed study, Paulus and colleagues reported crystallographic and solution structures of the salinomycin-sodium complexes in DMSO [11]. 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. The salinomycin-sodium complexes shows four oxygens of salinomycin including 1a-*O*, 11-*O*, 21-*O* and 25-*O* coordinate the central sodium atom, forming a relatively fixed conformation (Fig. 1). These results can explain why the modifications on different groups lead to different biological activities changes. For example, the salinomycin methyl ester showed at least 40 folds lower activities than salinomycin because its 1a-*O* can not coordinate with alkali metal ion. By contrast, the C20 hydroxyl acylated analogs showed much better activities because the 20-hydroxy group did not participate in the formation of the salinomycin-sodium complexes and the acylation modification can enhance membrane permeability.

Our group has synthesized several salinomycin C17, C20 and C21-diastereoisomers, and we found 17,21-*epi*-salinomycin showed similar biological activities but 17-*epi*-sailinomycin showed significantly reduced activities, indicating the conformations of salinomycin are crucial for biological activity [8]. However, compared to 6-6-5 spiro-ketal structure in salinomycin, which forms a relatively fixed conformation in the molecule, the C1-C12 fragment is much more flexible, leading to the variability of conformation of the whole molecule.

By analyzing the crystal structure of salinomycin combined with

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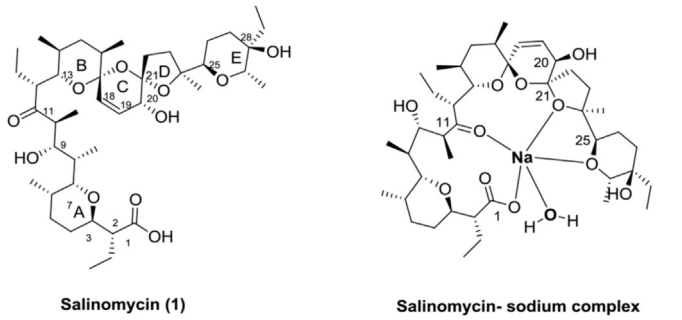


Fig. 1. Structure of salinomycin and salinomycin-Na complex (Schematic diagram).

sodium ions (structures I and II) and its derivatives, we found that the distance from the C1 to C20 atoms in salinomycin, 20-oxo-salinomycin, 20-deoxy-salinomycin and 20-epimer are usually 4–6.5 Å, but the corresponding distance in C1-substituted derivatives are usually greater than 7.5 Å (Table 1). Meanwhile, C20-modified compounds show good pharmacological activities, but the C1-modified compounds are substantially inactive, indicating the distance between C1 and C20 atoms is of great help in maintaining the active conformation.

Therefore, we designed and synthesized two conformationally restricted salinomycin derivatives by tethering the hydroxyl groups at C1 and C20 with different chain length, which can be expected to adopt compact conformations in the ion-binding process. Although usually C1-oxygen atom will participate in the binding of sodium ions, we hope that the appropriate conformation can make up for this loss.

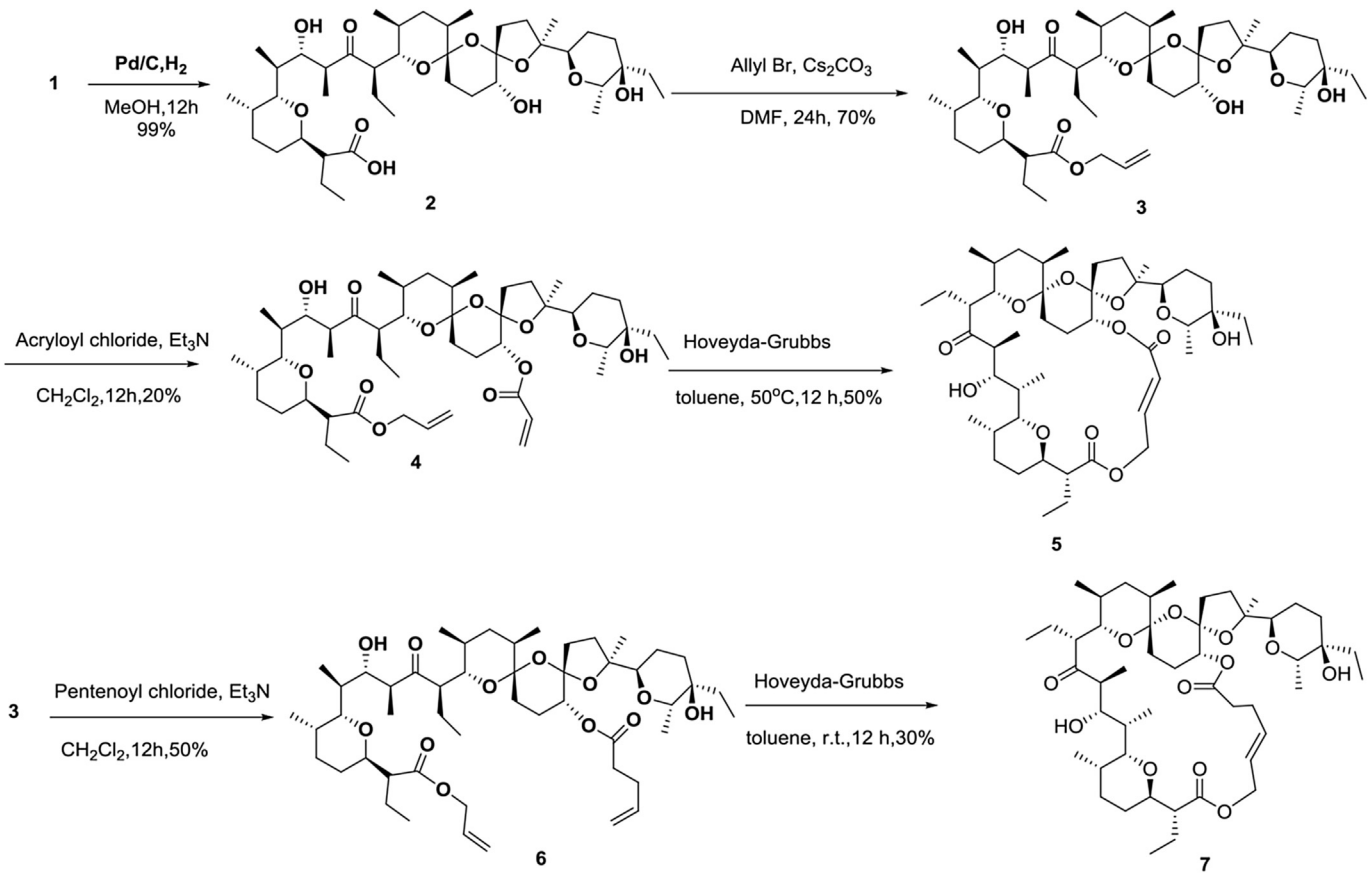
2. Results and discussion

2.1. Chemistry

We used ring closing metathesis reactions (RCM) to synthesize the conformationally restricted salinomycin derivatives, because the reactions are effective and widely applicable in the synthesis of macrocyclic compounds (see Scheme 1) [14]. Firstly, 18,19-dihydro salinomycin **2** was prepared by Pd/C catalytic hydrogenation of

Table 1
The distance of C1-C20 in X-ray structure of salinomycin and derivatives.

X-ray structure	Distance	X-ray structure	Distance
SAL-Na complex [11]	4.73(I), 6.34(II)	SAL-n-butyl amide [5a]	8.17
20-oxo-SAL-Na complex [12]	4.55	SAL-prop amide [6b]	8.39
20-deoxy-SAL-Na complex [13]	5.26	SAL-hydroxamic acid [5e]	8.03
20-*epi*-triazol-SAL-Na complex [8c]	5.40	SAL-*p*-iodophenacyl ester [4b]	7.79



Scheme 1. Synthesis of conformationally constrained salinomycin derivatives.

salinomycin to avoid the possible side reaction with the C18-19 double bond in RCM reaction, and the reduction of this double bond has little influence on the biological activity according to the literature [9a]. Then the C1 carboxylic group was esterified with bromopropene to give allyl ester **3** in 50% yield. The substrates **4** and **6** of RCM were synthesized by selective acrylation and 4-pentenoyl acylation to C20-hydroxyl groups of salinomycin allylic ester, respectively. For compound **4**, the treatment of allyl ester with acryloyl chloride and triethylamine in dichloromethane only gave desired product in about 20% yields, accompanied with the polymer as major by-product. We used 2D NMR to determine the structure, and δ 4.68 ppm was assigned to 20-H and δ72.76 ppm was assigned to 20-C. Similarly, pentenoyl acylated derivative **6** was prepared in 50% yield by the treatment of allyl ester **3** with pentenoyl chloride and triethylamine. Hoveyda − Grubbs catalyst was applied for the ring-closing metathesis reaction [15]. Treatment of compound **4** with 0.15 equiv catalyst in toluene at 50 °C for 12 h gave only *trans*-cyclic product **5** (J = 11.2 Hz) in 50% yield. *Trans*-cyclic product **7** was prepared by treatment of compound **6** with 0.07 equiv catalyst in toluene at room temperature for 12 h in 30% yield. Increase of the amount of catalyst to 0.15 equiv did not improve the yield significantly. The structures of cyclic product **5** and **7** were determined by 2D NMR spectrum analysis.

2.2. Antiproliferative activity

The antiproliferative activities of conformationally constrained salinomycin derivatives were evaluated in HT-29 colorectal cancer (CRC) cells [6b]; [8a], using MTT assay (Table 2). Sal reduces the CD133 + subpopulation of human CRC cells and inhibits Wnt signaling [16]; [17]. The effect of Sal on CRC has been further explained by induction of autophagy and accumulation of reactive oxygen species [18]; [19]. As expected, C1 modified derivatives **4** and **6** were inactive, just as the other C-1 modified compounds, but the cyclic derivatives **7** and **5** showed better biological activities, suggesting the relatively constrained conformation could make up for a biological activity loss caused by the modification on C1. Although cyclic derivative **5** was almost inactive, cyclic derivative **7** still showed some reduced activities compared with salinomycin, indicating it may adopt more appropriate conformations for the coordinate with alkali metal ion.

Table 2
Antiproliferative activities of the conformationally constrained salinomycin derivatives.

Compound	Inhibition ratio at 10 μM	IC_{50} (μM)
Sal(1)	76.1%	1.43
4	<10%	–
5	13.6%	>10
6	<10%	–
7	67.0%	7.64

2.3. Conformation analysis

According to crystal structures, the relative orientation of ring E and ring A is critical for the combination of alkali metal, typically, the distances of H3 and H25 are usually greater than 7.5 Å in the salinomycin as well as its C20-modified derivatives, but the distances of H3 and 28-OH are less than 6.0 Å (Tables 3 and 4). In contrast, C1-modified derivatives show the opposite trend, thus the distances of H3 and H25 in C1-modified derivatives are less than 6.5 Å, but the distances of H3 and 28-OH are greater than 8.0 Å. Based on these considerations, we then analyzed the conformation of the two cyclic salinomycin derivatives by ROESY spectrum in DMSO-d_6 [10]; [11]. The NOE effect was observed in H3 and H25 in compound **5**, indicating the two hydrogen atoms are close to each other (the distance < 5 Å, see supporting information), just as above C1-modified derivatives. It suggested the conformation of cyclic compound **5** is not suitable for the combination ability of alkali metal ion, so it showed the poor pharmacological activity. For compound **7**, neither of the NOE effect between H3 and H25 or H3 and 28-OH was observed, indicating the connective chain possibly restricted the similar conformation with salinomycin.

3. Conclusion

In summary, we have designed and synthesized two conformationally restricted salinomycin derivatives by tethering the hydroxyl groups at C1 and C20 with 4- or 6-membered atoms chain through ring closing metathesis reactions. The cyclic derivatives showed better biological activities than C1/C20 modified derivatives, indicating the importance of the compact conformation for the ion binding capacity. In addition, the length of the connective chain plays critical role in the biological activities, thus the cyclic derivative **7** preserved some pharmacological activity but derivative **5** with two carbon atom shorter chain showed significantly reduced activity. The conformations of the two cyclic salinomycin derivatives were analyzed by ROESY spectrum in DMSO-d_6, suggesting derivative **7** may adopt more appropriate conformations for the coordinate with alkali metal ion than derivative **5**, which has a closer distance between H3 and H25. Further studies on these conformationally restricted salinomycin derivatives are currently under way.

Table 3
The distance of H3-H25 in X-ray structure of salinomycin and derivatives.

X-ray structure	Distance	X-ray structure	Distance
SAL-Na complex [11]	8.57(I), 7.63(II)	SAL-n-butyl amide [5a]	6.28
20-oxo-SAL-Na complex [12]	8.09	SAL-prop amide [6b]	6.13
20-deoxy-SAL-Na complex [13]	7.99	SAL-hydroxamic acid [5e]	6.38
20-*epi*-triazol-SAL-Na complex [8c]	8.47		

Table 4
The distance of H3-OH28 in X-ray structure of salinomycin and derivatives.

X-ray structure	Distance	X-ray structure	Distance
SAL-Na complex [11]	5.47(I), 4.37(II)	SAL-n-butyl amide [5a]	9.44
20-oxo-SAL-Na complex [12]	3.59	SAL-prop amide [6b]	9.23
20-deoxy-SAL-Na complex [13]	4.50	SAL-hydroxamic acid [5e]	9.39
20-*epi*-triazol-SAL-Na complex [8c]	4.70	SAL-p-iodophenacyl ester [4b]	>8.17

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.06.063.

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