Bioorganic & Medicinal Chemistry Letters 27 (2017) 1624–1626

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological activity of salinomycin-hydroxamic acid conjugates

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ARTICLE INFO

Article history:
Received 18 October 2016
Revised 23 December 2016
Accepted 26 January 2017
Available online 28 January 2017

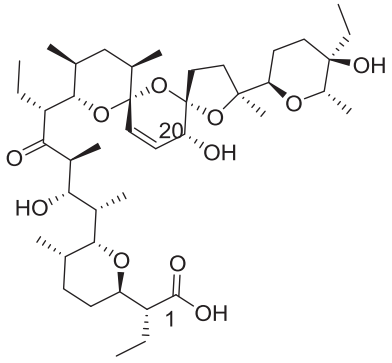
Keywords:
Salinomycin
Hydroxamic acid
Conjugates
Anti-cancer

ABSTRACT

Several salinomycin-hydroxamic acid conjugates were designed and synthesized. Most conjugates showed better antiproliferative activities than salinomycin in HT-29 colon cancer, HGC-27 gastric cancer, and especially in MDA-MB-231 triple-negative human breast cancer cells. These conjugates are stable in cell culture media, and they showed much better biological activities than the 1:1 physical mixture with hydroxamic acids and salinomycin. The better membrane permeability and hydrolysis rate of the conjugates may lead to the activity improvements.

Salinomycin (**1**, Fig 1) is a kind of polyether antibiotics isolated from *Streptomyces albus*, which can transfer alkali metal ions such as Na^+ and K^+ through cytomembrane.[1] It has been used in broiler batteries and other livestock as an anticoccidial drug and growth promoters for many decades.[2] Recent studies proved salinomycin also effectively kills cancer stem cells (CSCs) and differentiated cancer cells that display efficient mechanisms of resistance to cytotoxic drugs and radiation, including leukemia, breast cancer, gastric cancer, lung adenocarcinoma, osteosarcoma, colorectal cancer, squamous cell carcinoma, prostate cancer *in vitro* and *in vivo*.[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]

To study the structure–activity and structure–toxicity relationship of salinomycin, medicinal chemists have synthesized several C1 and C20-modified salinomycin derivatives, some of which showed better antitumor activities.[5–15] Daniel Strand's group synthesized several C20 hydroxyl acylated salinomycin analogs, which efficiently decreased the CSC population at a 50 nM concentration.[6,16] Their following study found the anti-cancer activity of



Salinomycin (1)

Fig. 1. The structure of salinomycin.

C20-deoxy-saliomycin is reduced, which emphasizes the importance of substitution at C20 for the activity.[8] Our group synthesized several salinomycin diastereoisomers and their acylated derivatives, and we found the stereochemistry has important influences on the biological activities.[17,18] These results together suggest that the alkali metal ion transport ability of salinomycin is crucial to its biological activities.

Compared to C20-modified derivatives, which showed obviously higher pharmacological activities, the C1-ester, amide and

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http://dx.doi.org/10.1016/j.bmcl.2017.01.080
0960-894X/

conjugates with other active substances such as amino acid,[11] floxuridine[12] and silybin[13] almost showed much lower antitumor activities. A possible explanation is that extra steric hindrance on the head (C1) affects the combination and transport of the sodium ions, because salinomycin need form "head to tail" type of intramolecular hydrogen bonds as a pseudo-cyclic structure.[19] Nevertheless, some C1-modified salinomycin derivatives which have a readily hydrolyzed connecting bond showed better antitumor activities such as compound **2**[9] and **3**[5] (Fig 2).

Different from ester and amide, carboxylic acid and hydroxamic acid conjugates are relatively more easily to be hydrolyzed. In additions, some hydroxamic acids also have ion-binding ability, which provides central functionality in a number of metalloproteinase inhibitors, such as histone deacetylase inhibitors vorinostat and panobinostat.[20] Coupling the salinomycin with hydroxamic acid should be an interesting attempt. We hope these conjugates can improve the membrane permeability and antitumor activities.

To study the effect of the substituent groups, ten hydroxamic acids **4a–13a** were purchased or prepared from N,N′-carbonyldiimidazole (CDI) activated carboxylic acids and unprotected hydroxylamine as Usachova's report,[21] which were directly coupled with salinomycin at carboxy group by the dicyclohexylcarbodiimide (DCC), affording salinomycin-hydroxamic acid conjugates **4b–13b** (Scheme 1). The ^{1}H NMR (~11.0 ppm, an intramolecular hydrogen bond with N—H and C═O(C1)), ^{13}C NMR and HRMS-ESI confirmed the structures of the conjugates and the X-ray of the monocrystal **11b** in CH_3CN clearly demonstrated the three-dimensional structure (CCDC 1490679, Fig 3).

The antiproliferative activities of salinomycin-hydroxamic acid conjugates were evaluated in HT-29 colorectal cancer, HGC-27 gastric cancer and triple-negative MDA-MB-231 breast cancer cells using MTT assay (Table 1). These conjugates were proved to be stable for 72 h in cell culture media. Most conjugates showed better antiproliferative activities than salinomycin against these cancer cell lines, especially in MDA-MB-231 cell. Acetyl and 2, 4-

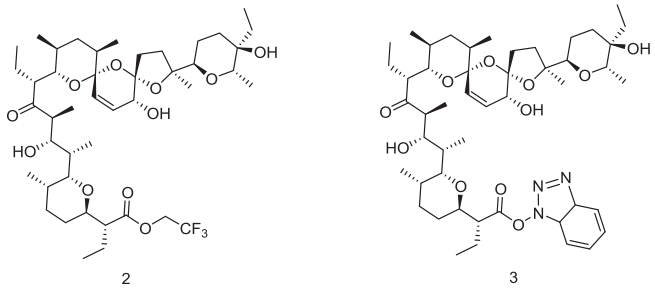


Fig. 2. The structure of salinomycin derivatives.

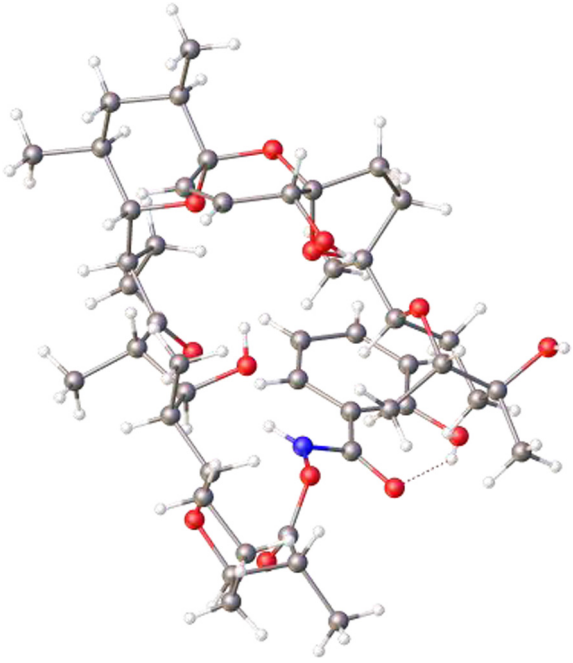
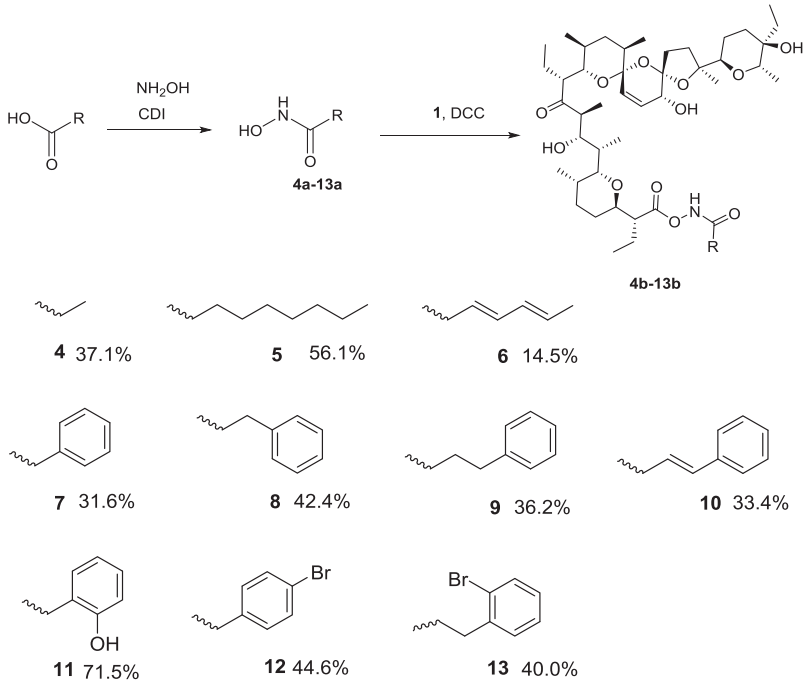


Fig. 3. The X-ray structure of salinomycin-salicylhydroxamic acid conjugate (**11b**).



Scheme 1. The synthesis of the salinomycin-hydroxamic acid conjugates.

Table 1
Antiproliferative activities in three cancer cell lines (IC_{50}, [μM][a]).

Compd	HT-29	HGC-27	MDA-MB-231
Sal	1.43 ± 0.47	2.57 ± 0.12	6.91 ± 0.76
4b	2.32 ± 0.22	2.25 ± 0.08	3.79 ± 0.48
5b	0.96 ± 0.02	1.35 ± 0.39	2.69 ± 0.16
6b	2.31 ± 0.26	2.63 ± 0.08	3.82 ± 1.13
7b	0.85 ± 0.02	0.92 ± 0.04	2.26 ± 0.05
8b	0.90 ± 0.06	1.29 ± 0.34	1.01 ± 0.02
9b	0.80 ± 0.10	1.05 ± 0.15	1.89 ± 0.24
10b	7.23 ± 1.39	7.27 ± 0.88	9.64 ± 0.02
11b	0.88 ± 0.05	0.92 ± 0.01	2.78 ± 0.25
12b	0.70 ± 0.13	0.51 ± 0.21	0.94 ± 0.04
13b	0.80 ± 0.01	0.76 ± 0.08	0.93 ± 0.04

[a] IC_{50} values are the mean (±SE) for 50% reduction of MTT compared to control. For all entries, n = 3.

Table 2
Antiproliferative activity in MDA-MB-231 cell lines (IC_{50}, [μM]).

Compd	MDA-MB-231	Compd	MDA-MB-231
Sal	6.53	**1+10a**	8.98
1+6a	7.89	**1+12a**	>10.0
1+7a	9.85	**1+13a**	>10.0
1+8a	9.72	**Taxol**	0.08

hexadiene acyl derivatives (**4b** and **6b**) showed comparative activities with salinomycin, while octanoyl derivative (**5b**) is a little more potent (1.5–2.5 fold higher than salinomycin). Compounds **7b–9b** with 0–2 carbons between carbonyl and phenyl group showed similar activities as octanoyl derivative (**5b**) (1.5–7 fold higher than salinomycin), indicating the length of the carbon chain has little influence on the biological activities. Salicyloyl derivative (**11b**) showed comparative activities with benzoyl derivative (**7b**), but brominated products (**12b** and **13b**) were more potent, especially compound **12b** showed 7-fold better activities in HGC-27 gastric cancer and MDA-MB-231 triple negative human breast cancer cells. However, the cinnamoyl derivative with an unsaturated bond (**10b**) showed the reduced activities.

Then some hydroxamic acids as well as the 1:1 mixture with salinomycin were assayed in MDA-MB-231 cell lines. All the tested hydroxamic acids did not show antiproliferative biological activities at 10 μM. The physical mixture performed weaker biological activities than single salinomycin (Table 2). These results indicated salinomycin and simple hydroxamic acids together did not show a potent synergistic effect in tumor cells. By contrast, our salinomycin-hydroxamic acid conjugates showed better biological activities, especially in MDA-MB-231 cell lines, possibly because they have better membrane permeability and could be readily cleaved to release salinomycin. It seems that the efficacy of these compounds are due to their membrane permeability and hydrolysis rate of linkage within cells. Octanoyl derivative (**5b**) has a longer carbon chain than Acetyl derivative (**4b**), and the former is more potent. Among these derivatives, the phenyl substituted compounds especially which have electron-withdrawing group (**12b** and **13b**) could be cleaved faster after they get inside the cells, and they all showed better antiproliferative activities. The derivatives including conjugated double bond (**6b** and **10b**) are relatively harder to be cleaved, so they showed weaker activities.

In summary, we have synthesized and evaluated the biological activities of several salinomycin-hydroxamic acid conjugates. Most conjugates showed better antiproliferative activities than salinomycin in HT-29 colon cancer, HGC-27 gastric cancer, and especially in triple-negative MDA-MB-231 human breast cancer cells. These conjugates are stable in cell culture media, and they showed much better biological activities than the 1:1 physical mixture with hydroxamic acids and salinomycin. The better membrane permeability and hydrolysis rate of the conjugates lead to the activity improvements. Further studies on these conjugates are currently under way.

Acknowledgment

We thank The National Natural Science Fund (81602958), Beijing Natural Science Foundation (7164282), the Fundamental Research Funds for the Central Universities (2016ZX350006) and 973 program (2015CB964800) for financial support.

A. Supplementary material

The preparation and the characterization of the compounds **4b–13b,** as well as the methods of the cell assay and the stability study. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.01.080.

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