Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d1ob02292j

Synthesis and anti-tumor activity evaluation of salinomycin C20-*O*-alkyl/benzyl oxime derivatives†

Bo Li, ‡§[a] Jun Wu,‡[b,c,d] Lei Tang,‡[e] Xu Lian,[a] Zhongwen Li,[b,c,d] Wenfang Duan,[e] Tong Qin,[a] Xintong Zhao,[a] Yuhua Hu,[a] Chi Zhang,[a] Tianlei Li,[a] Jie Hao,[b,c,d] Wenxuan Zhang, *[a] Jihong Zhang*[e] and Song Wu*[a]

Received 23rd November 2021,
Accepted 14th December 2021

DOI: 10.1039/d1ob02292j

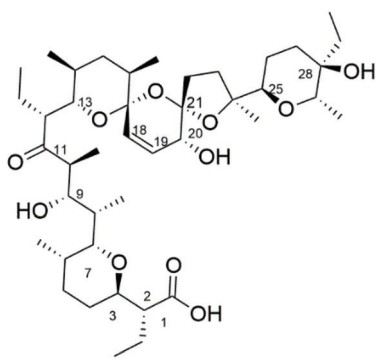
rsc.li/obc

Seventeen C20-*O*-alkyl/benzyl oxime derivatives were synthesized by a concise and effective method. Most of these derivatives showed tens to several hundred nanomolar IC_{50} values against HT-29 colorectal, HGC-27 gastric and MDA-MB-231 breast cancer cells, whose antiproliferative activity is 15–240 fold better than that of salinomycin. The C20-oxime etherified derivatives can coordinate potassium ions, and further adjust the cytosolic Ca^{2+} concentrations in HT-29 cells. The significant improvement of the potency should be attributed to the better ion binding and transport ability of the modified derivatives. In addition, the C20-*O*-alkyl/benzyl oxime derivatives showed much better selectivity indexes (SI) than salinomycin, indicating that they present lower neurotoxic risk.

1. Introduction

Salinomycin (**1**, Fig. 1), a Na^+/K^+ ionophore, is a kind of polyether antibiotic isolated from *Streptomyces albus*, which exhibits a large spectrum of anticoccidial, antimicrobial, antifungal and antiviral activities.[1] Recent studies have shown that salinomycin is able to reduce the proportion of cancer stem cells (CSCs), kill differentiated cancer cells, and inhibit tumor growth in animal models,[2] making salinomycin a potential anti-cancer agent. Nevertheless, the inherent neurotoxic side effect of salinomycin has impeded its application in clinical use.[3]

The metal cation binding and transport ability is the molecular basis for the inhibition of stem-like cancer cells by salinomycin and other ionophores.[4,5] Recently, Rodriguez and co-workers have revealed that salinomycin and its derivatives kill breast cancer stem cells *via* lysosomal iron targeting.[6] In response to the ensuing cytoplasmic depletion of iron, the cells triggered the degradation of ferritin in lysosomes, leading to further iron loading in this organelle. The iron-mediated production of reactive oxygen species promoted lysosomal membrane permeabilization, activating a cell death pathway consistent with ferroptosis. Strand and co-workers have proved the induction of Ca^{2+} release from the endoplasmic reticulum (ER) into the cytosol, presumably by mediating a counter-flux



Salinomycin (1)

Fig. 1 The structure and atom number of salinomycin.

[a]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China. E-mail: wxzhang@imm.ac.cn, ws@imm.ac.cn*

[b]*State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*

[c]*Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China*

[d]*National Stem Cell Resource Center, Chinese Academy of Sciences, Beijing 100101, China*

[e]*Medical School of Kunming University of Science and Technology, Kunming, 650031, China. E-mail: Zhjihong2000@kust.edu.cn*

†Electronic supplementary information (ESI) available. CCDC 1465419. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1ob02292j

‡These authors contributed equally to this work.

§Present address: Université de Bordeaux, CNRS, CBMN, UMR-5248, Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac (France).

of K^+ ions.[7] Salinomycin-induced ER Ca^{2+} depletion up-regulates C/EBP homologous protein (CHOP), further inhibiting Wnt signaling by down-regulating β-catenin. The increased cytosolic Ca^{2+} also activates protein kinase C, which has been shown to inhibit Wnt signaling. The previous structure–activity relationship (SAR) of salinomycin diastereoisomers and conformationally restricted derivatives reported by our group has also emphasized the importance of the ion binding and transport ability of salinomycin to its biological activity.[8]

To date, several salinomycin C1-esters,[9] C1-amides,[10] salinomycin–hydroxamic conjugates,[11] and C1-conjugates with other active biomolecules,[12] as well as dimers of salinomycin[13] were synthesized and evaluated in antiproliferative models. In spite of all the efforts, only a few of the C1-modified salinomycin derivatives improve the antiproliferative activity and selectivity to cancer cells, possibly because the C1-carboxyl group of salinomycin is directly involved in the ion-binding according to the structure of the salinomycin-Na^+ complex, in which the O_1, O_{11}, O_{21} and O_{25} atoms combine with the sodium ion (Fig. 2).[14]

However, the C20-hydroxyl group does not combine with the metal cations, so modification on this site usually does not disturb the ion binding ability. According to Strand's work, most of the alkyl or aromatic C20-*O*-esters, C20-*O*-carbamates and C20-*O*-carbonates exhibited more than five times better inhibitory activity than salinomycin.[15] In addition, Rodriguez's group reported C20-*N*-amino derivatives showing nanomolar activity against breast CSCs.[6,16] When the configuration of C20 was inverted, we also observed semblable SAR results, such as the C20-*epi*-*O*-esters reported by our group and the C20-*epi*-*N*-amides, C20-*epi*-*N*-carbamates and C20-*epi*-triazoles reported by Jiang's group.[17,18] Therefore, the modification of the C20-hydroxyl group is an attractive strategy for the structural optimization of salinomycin.

Modifications by oxime ether are a successful strategy in natural and synthesized biologically active compounds, including antibacterial and antitumor agents, like clinically used cefixime and roxithromycin.[19] In addition, the biologically potent oxime ether is easy to prepare and chemically stable.

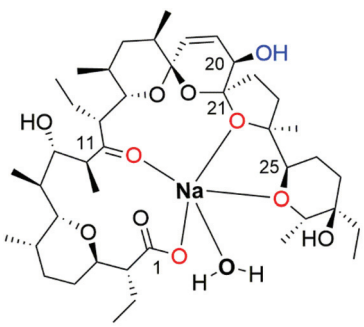


Fig. 2 Salinomycin–sodium complex (O_1, O_{11}, O_{21} and O_{25} are highlighted in red and the C20-hydroxyl group is highlighted in blue).

Based on C20-oxo-salinomycin (**2**), which is slightly more potent than salinomycin, several C20-*O*-alkyl/benzyl oxime derivatives were synthesized. We hoped that C20-*O*-alkyl/benzyl oxime derivatives can improve the biological activity and selectivity of salinomycin.

2. Results and discussion

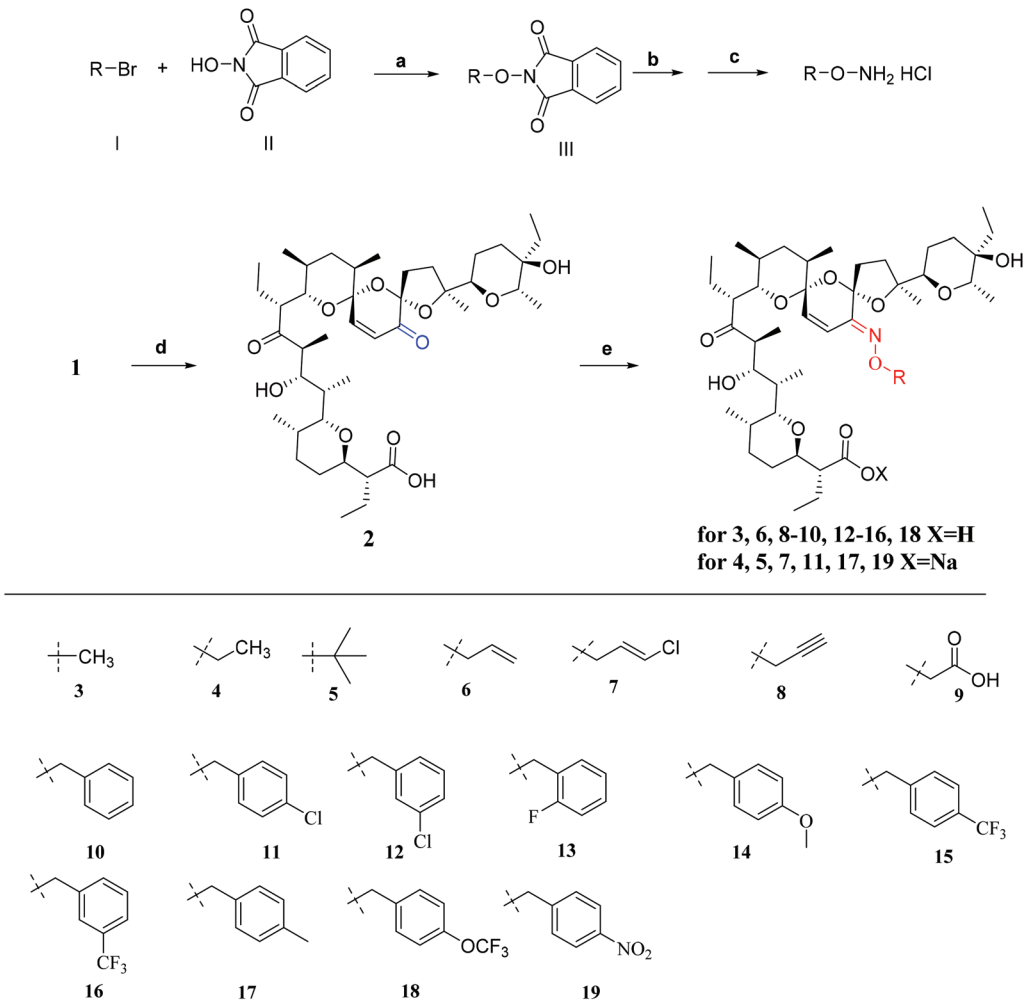
2.1. Chemistry

The *O*-alkyl/benzyl hydroxylamines were purchased from commercial sources and benzyl hydroxylamines were prepared following the reported methods (Scheme 1).[20] Alkylation of *N*-hydroxyphthalimide (NOP, II) with benzyl bromide (I) gave coupled intermediates III in the presence of potassium carbonate, which were treated with hydrazine hydrate and then hydrochloride to generate hydroxylamine hydrochlorides. The allylic C20 hydroxyl group of salinomycin was oxidized by 4 equiv. of Dess–Martin periodinane (DMP) to give the corresponding C20-oxo-salinomycin (**2**) in about 60% yield and C9/C20-*di*-oxo-salinomycin in 10% yield, by controlling the reaction time within 30 min at room temperature. Finally, the hydroxylamine reacted with the C20 ketone (**2**) at room temperature for 4–12 hours to afford C20-*O*-alkyl/benzyl oxime derivatives (**3–19**) in 30–60% yields after purification by column chromatography on silica gel. The structure of the synthesized derivatives was confirmed by ^{1}H NMR, ^{13}C NMR and HR-ESI mass analyses.

The single crystal of *O*-benzyl oxime derivative **10** (solvated acetonitrile molecule, CCDC 1465419†) was cultured in acetonitrile and the 3D structure was characterized using X-ray single crystal diffraction (Fig. 3, Table S1†). The result showed that the benzyl oxime ether group takes the *E*-configuration to relieve the steric hindrance from rings D and E of salinomycin, and it is far away from the O_1–O_{11}–O_{21}–O_{25} cation binding region. In addition, the molecule still adopted the 'head-to-tail' conformation such as with salinomycin, which was stabilized by the additional three intramolecular hydrogen bonds between O_{1A} and O_9, O_{1B} and O_{28} and O_{11} and O_{28}, indicating that it retained the ion binding and transport ability.[21] The ability of compound **10** to coordinate the potassium ion was further examined by ^{1}H NMR experiments. When potassium chloride (KCl) was added to CD_3OD, the chemical shifts of the protons (between 2.5 and 4.0 ppm) including H-2, H-10, H-12, H-25 and H-29 (protons near O_1, O_{11}, O_{21} and O_{25} atoms, Table S2†) in oxime etherified derivative **10** changed significantly, suggesting the change of the conformation of the derivative **10**-K^+ complex from the free molecule; thus we deduced that these compounds can also coordinate potassium ions (Fig. 4).

2.2. Biological test

The antiproliferative activities of the C20-*O*-alkyl/benzyl oxime derivatives were evaluated in HT-29 colorectal cancer, HGC-27 gastric cancer and triple negative MDA-MB-231 human breast cancer cells by the MTT assay



Scheme 1 The route for the synthesis of oxime etherified salinomycin derivatives.

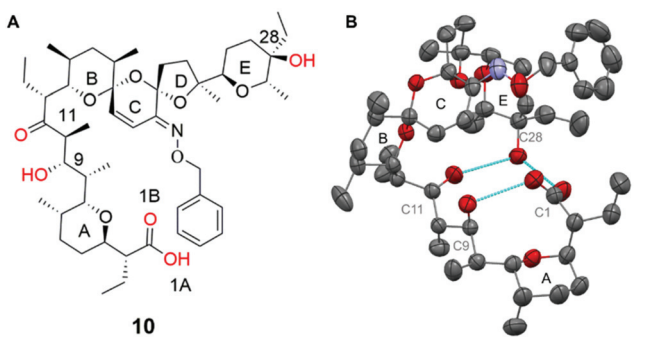


Fig. 3 Chemical structure (A) and X-ray structure shown as thermal ellipsoids (B) of compound **10**. The intra hydrogen bonds are represented with a cyan dashed line (B) and the oxygen atoms that form hydrogens bonds are highlighted in red (A).

(Table 1). Most of the C20-oxime etherified derivatives (except for **9**) exhibited much better antiproliferative activities up to 15–240 fold than that of salinomycin, with IC_{50} values ranging from dozens to several hundred nanomolar against HT-29 colorectal cancer and HGC-27 gastric cancer cell lines, as well as MDA-MB-231 breast cancer cells. Although C20-oxo-salinomycin is also more active than the natural structure (**2**, Table 1), the alkyl (**3–8**) and benzyl (**10–19**) oxime etherified derivatives also exhibited about 10 fold better potencies than C20-oxo salinomycin **2**, indicating that the etherified group is beneficial for the antiproliferative activity. Compound **9** with the carboxyl group basically lost its biological activity, possibly because the extra carboxyl group interfered with the ion binding and transport ability.

However, there was no obvious difference in the biological activity intensity between the *O*-alkyl and *O*-benzyl oxime derivatives against the HT-29 cell line, and most of the com-

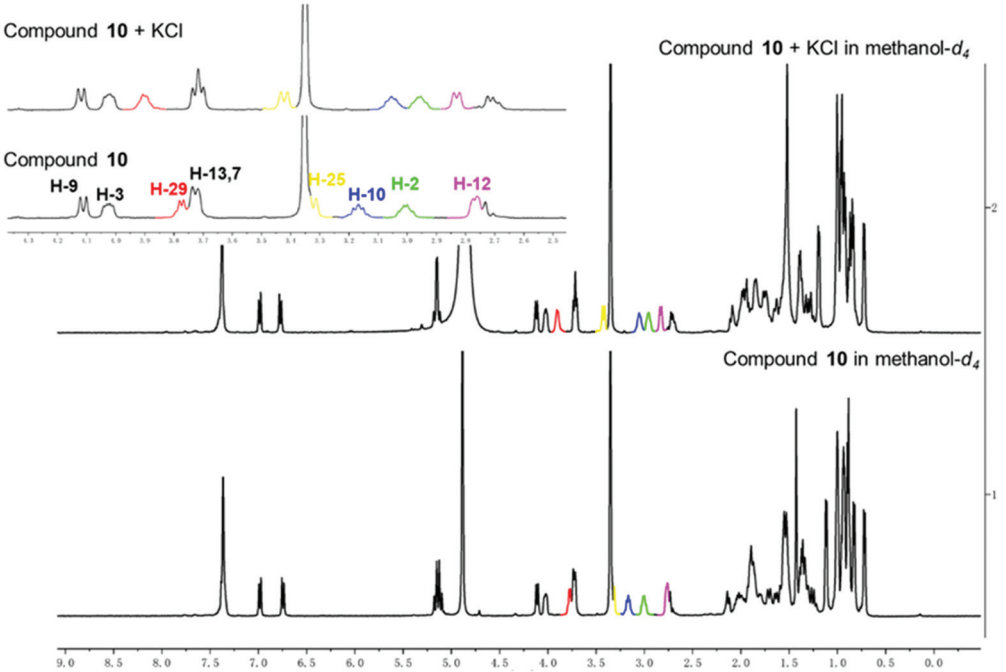


Fig. 4 ^{1}H NMR spectra of compound **10** with and without KCl in methanol-d_4. The shifted proton peaks between 4.5 and 2.5 ppm due to ion binding are labelled and highlighted in different colors.

Table 1 Antiproliferative activities of the salinomycin derivatives (IC_{50}, μM)

Compound	HT-29	HGC-27	MDA-MB-231
1	2.722	2.457	6.907
2	0.373	0.563	0.935
3	0.050	0.030	0.090
4	<0.03	0.087	0.066
5	<0.03	0.086	0.092
6	<0.03	0.043	0.037
7	<0.03	0.093	0.087
8	<0.03	0.092	0.085
9	>10	>10	>10
10	<0.03	0.026	0.122
11	0.065	0.030	0.402
12	0.035	0.024	0.306
13	<0.03	0.087	0.067
14	0.036	0.027	0.097
15	0.037	0.022	0.184
16	<0.03	0.094	0.040
17	0.051	0.029	0.142
18	0.027	0.010	0.109
19	0.046	0.028	0.177

pounds showed less than 30 nM IC_{50} values (about 100 fold more potent than salinomycin). In the HGC-27 gastric cancer cell line, most of the benzyl derivatives showed better antiproliferative activity than the alkyl derivatives, and derivative **18** was the most potent compound with an IC_{50} value of only 10 nM (about 240 fold more potent than salinomycin). In contrast, the alkyl derivatives were relatively more potent than benzyl derivatives in the MDA-MB-231 cancer cell line, and the allyl derivative **6** was the most potent compound with a 37 nM IC_{50} value (about 180 fold more potent than salinomycin).

The potential neurotoxic side effect of salinomycin is a difficult issue.[3] We tested the inhibitory effect of salinomycin and its oxime ether derivatives against the human neuroblastoma SH-SY5Y cell line at a concentration of 1 μM (Table S3†).[22] The inhibitory IC_{50} value of salinomycin against SH-SY5Y cells is 1 μM, which is lower than those of HT-29, HGC-27 and MDA-MB-231 cancer cells. To our delight, no significant toxicity effect was observed from most of the oxime etherified derivatives except three compounds (**11**, **15** and **18**), indicating better selectivity index (SI) values of the derivatives and lower neurotoxic risk than those of salinomycin. It is worth noting that compound **6** showed excellent cytotoxicity and good selectivity with SI > 20 to all three tested cancer cell lines.

To investigate whether the C20-oxime etherified derivatives possess the same mechanism as salinomycin reported by Strand and co-workers, we first used flow cytometry to verify whether the derivative can disrupt the intracellular ion homeostasis.[7] As illustrated in Fig. 5a, HT29 cells were incubated with salinomycin and **6** for 24 h, and then stained with the calcium indicator Fluo-3 AM. The flow cytometry analysis showed that both salinomycin and **6** increased the cytosolic Ca^{2+} concentration in HT-29 cells, but the latter was more potent even at a dose 10-fold lower than that of salinomycin (0.5 μM *vs.* 5 μM). Since the ER is a Ca^{2+} storage organelle, we then explored whether the elevated Ca^{2+} source in the cytosol originates from the ER. HT29 cells were incubated with 5 μM salinomycin and 0.5 μM **6** for 24 h, and then stained with the calcium indicator Fluo-4 AM. After that, we used thapsigargin, an inhibitor of the sarcoplasmic-ER Ca^{2+} ATPase, and ATP, a

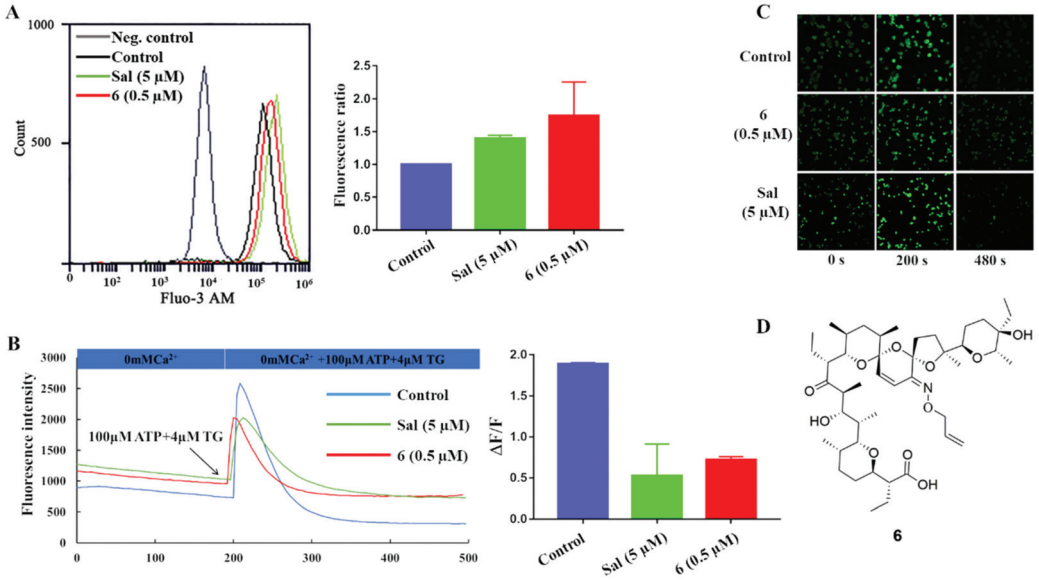


Fig. 5 Salinomycin and compound **6** improve the cytosolic Ca^{2+} concentration. (A) HT-29 cells were treated with salinomycin (5 μM) and **6** (0.5 μM) for 24 hours. The cytoplasmic Ca^{2+} concentration was examined by flow cytometry. (B) After depletion of ER Ca^{2+} stores with thapsigargin (TG), ER stored Ca^{2+} release was examined by confocal microscopy upon salinomycin (5 μM) and **6** (0.5 μM) treatments. (C) Representative confocal images of the baseline (0 s) and maximum fluorescence (200 s) of Fluo-4 AM to reflect the Ca^{2+} released from the ER to the cytosol in HT29 cells. (D) The chemical structure of compound **6**.

calcium releasing agonist, to deplete Ca^{2+} stored in the ER. We then used laser confocal microscopy to record the basal Ca^{2+} fluorescence intensity in the cytoplasm (0 s) and the maximum Ca^{2+} fluorescence intensity when the ER Ca^{2+} concentration was 0 (200 s) (Fig. 5b and c). As shown in Fig. 5b, the Ca^{2+} release from the ER decreased after salinomycin and **6** treatment, suggesting that salinomycin and **6** caused a reduction of Ca^{2+} storage in the ER. Meanwhile, the basal Ca^{2+} fluorescence intensity in the cytoplasm increased. Overall, these results suggest that salinomycin and **6** induced Ca^{2+} release from the ER into the cytoplasm. Taken together, our results indicate that the C20-oxime etherified derivative **6** exhibited more potency than salinomycin as an ion carrier.

3. Conclusion

In conclusion, we have synthesized seventeen salinomycin C20-*O*-alkyl/benzyl oxime derivatives by a concise and effective two-step routine. Most of the C20-oxime etherified derivatives exhibited much better antiproliferative activities up to 15–250 fold than that of salinomycin against HT-29 colorectal, HGC-27 gastric and MDA-MB-231 breast cancer cells, with dozens to several hundred nanomolar IC_{50} values. The modified derivatives can also coordinate potassium ions according to the NMR experiments. The C20-oxime etherified derivatives can elevate the cytosolic Ca^{2+} concentrations in HT-29 cells from the endoplasmic reticulum, indicating a similar mechanism to salinomycin. The improvements of the antiproliferative activity of the etherified derivatives are possibly due to their better ion binding and transport ability. In addition, the C20-*O*-alkyl/benzyl oxime derivatives showed much better selectivity indexes (SI) than salinomycin, indicating that they had lower neurotoxic risk. Further pharmacological evaluation of C20-oxime etherified derivatives is ongoing.

Author contribution

W. Zhang, J. Zhang and S. Wu designed and coordinated the project. B. Li and W. Zhang developed the hypothesis, and wrote and reviewed the manuscript. B. Li, W. Zhang, J. Wu, L. Tang, X. Lian, Z. Li, W. Duan, T. Qin, X. Zhao, Y. Hu, C. Zhang, T. Li and J. Hao performed the experiments and analytical measurements.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank the National Natural Science Foundation (81602958), Drug Innovation Major Project (2018ZX09711001-005) and 973 Program (2015CB964803) for financial support.

References

1 (*a*) Y. Miyazaki, M. Shibuya, H. Sugawara, O. Kawaguchi and C. Hirsoe, Salinomycin, a new polyether antibiotic,

J. Antibiot., 1974, **27**, 814–821; (*b*) B. C. Pressman, Biological applications of ionophores, *Ann. Rev. Biochem.*, 1976, **45**, 501; (*c*) D. A. Kevin II, D. A. Meujo and M. T. Hamann, Polyether ionophores: broad-spectrum and promising biologically active molecules for the control of drug-resistant bacteria and parasites, *Expert Opin. Drug Discovery*, 2009, **4**, 109–146.

2 (*a*) P. B. Gupta, T. T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R. A. Weinberg and E. S. Lander, Identification of selective inhibitors of cancer stem cells by high-throughput screening, *Cell*, 2009, **138**, 645–659; (*b*) C. Naujokat and R. Steinhart, Salinomycin as a drug for targeting human cancer stem cells, *J. Biomed. Biotechnol.*, 2012, **2012**, 950658.

3 W. Boehmerle and M. Endres, Salinomycin induces calpain and cytochrome c-mediated neuronal cell death, *Cell Death Dis.*, 2011, **2**, e168.

4 (*a*) B. Borgström, X. Huang, C. Hegardt, S. Oredsson and D. Strand, Structure–activity relationships in salinomycin: cytotoxicity and phenotype selectivity of semi–synthetic derivatives, *Chem. – Eur. J.*, 2017, **23**, 2077–2083; (*b*) M. Antoszczak, A comprehensive review of salinomycin derivatives as potent anticancer and anti-CSCs agents, *Eur. J. Med. Chem.*, 2019, **166**, 48–64.

5 F. Shen, S. Dai, N. Wong, S. Deng, A. Wong and D. Yang, Mediating K+ /H+ Transport on Organelle Membranes to Selectively Eradicate Cancer Stem Cells with a Small Molecule, *J. Am. Chem. Soc.*, 2020, **142**, 10769–10779.

6 T. T. Mai, A. Hamaï, A. Hienzsch, T. Cañeque, S. Müller, J. Wicinski, O. Cabaud, C. Leroy, A. David, V. Acevedo, A. Ryo, C. Ginestier, D. Birnbaum, E. Charafe-Jauffret, P. Codogno, M. Mehrpour and R. Rodriguez, Salinomycin kills cancer stem cells by sequestering iron in lysosomes, *Nat. Chem.*, 2017, **9**, 1025–1033.

7 X. Huang, B. Borgström, J. Stegmayr, Y. Abassi, M. Kruszyk, H. Leffler, L. Persson, S. Albinsson, R. Massoumi, I. G. Scheblykin, C. Hegardt, S. Oredsson and D. Strand, The molecular basis for inhibition of stemlike cancer cells by salinomycin, *ACS Cent. Sci.*, 2018, **4**, 760–767.

8 (*a*) W. Zhang, J. Wu, B. Li, H. Wu, L. Wang, J. Hao, S. Wu and Q. Zhou, Structure–activity & structure–toxicity relationship study of salinomycin diastereoisomers and their benzoylated derivatives, *Org. Biomol. Chem.*, 2016, **14**, 2840–2845; (*b*) W. Zhang, J. Wu, B. Li, X. Lian, J. Xia, Q. Zhou and S. Wu, Design and synthesis of conformationally constrained salinomycin derivatives, *Eur. J. Med. Chem.*, 2017, **138**, 353–356.

9 (*a*) M. Mitani, T. Yamanishi and Y. Miyazaki, Salinomycin: a new monovalent cation ionophore, *Biochem. Biophys. Res. Commun.*, 1975, **66**, 1231–1236; (*b*) H. Kinashi, N. Otake, H. Yonehara, S. Sato and Y. Saito, Studies on the ionophorous antibiotics. I. The crystal and molecular structure of salinomycin p-iodophenacyl ester, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1975, **31**, 2411–2415; (*c*) M. Antoszczak, K. Popiel, J. Stefańska, J. Wietrzyk, E. Maj, J. Janczak, G. Michalska, B. Brzezinski and A. Huczyński, Synthesis, cytotoxicity and antibacterial activity of new esters of polyether antibiotic - salinomycin, *Eur. J. Med. Chem.*, 2014, **76**, 435–444.

10 (*a*) A. Huczyński, J. Janczak, M. Antoszczak, J. Wietrzyk, E. Maj and B. Brzezinski, Antiproliferative activity of salinomycin and its derivatives, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 7146–7150; (*b*) M. Antoszczak, E. Maj, J. Stefańska, J. Wietrzyk, J. Janczak, B. Brzezinski and A. Huczyński, Synthesis, antiproliferative and antibacterial activity of new amides of salinomycin, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 1724–1729; (*c*) M. Antoszczak, E. Maj, A. Napiórkowska, J. Stefańska, E. Augustynowicz-Kopeć, J. Wietrzyk, J. Janczak, B. Brzezinski and A. Huczyński, Synthesis, anticancer and antibacterial activity of salinomycin N-benzyl amides, *Molecules*, 2014, **19**, 19435–19459; (*d*) B. Borgström, X. Huang, E. Chygorin, S. Oredsson and D. Strand, Salinomycin hydroxamic aids: synthesis, structure, and biological activity of polyether ionophore hybrids, *ACS Med. Chem. Lett.*, 2016, **7**, 635–640.

11 B. Li, J. Wu, W. Zhang, Z. Li, G. Chen, Q. Zhou and S. Wu, Synthesis and biological activity of salinomycin-hydroxamic acid conjugates, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 1624–1626.

12 (*a*) M. Antoszczak, M. Sobusiak, E. Maj, J. Wietrzyk and A. Huczyński, Synthesis and antiproliferative activity of new bioconjugates of Salinomycin with amino acid esters, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 3511–3514; (*b*) A. Huczyński, M. Antoszczak, N. Kleczewska, M. Lewandowska, E. Maj, J. Stefańska, J. Wietrzyk, J. Janczak and L. Celewicz, Synthesis and biological activity of salinomycin conjugates with floxuridine, *Eur. J. Med. Chem.*, 2015, **93**, 33–41; (*c*) M. Antoszczak, G. Klejborowska, M. Kruszyk, E. Maj, J. Wietrzyk and A. Huczyński, Synthesis and antiproliferative activity of silybin conjugates with salinomycin and monensin, *Chem. Biol. Drug Des.*, 2015, **86**, 1378–1386.

13 (*a*) M. Antoszczak, E. Maj, B. Borgström, S. Oredsson, A. Huczyński, J. Wietrzyk and D. Strand, Bivalent polyether ionophores: Synthesis and biological evaluation of C2-symmetric salinomycin dimers, *Tetrahedron Lett.*, 2017, **58**, 2396–2399; (*b*) M. Huang, Z. Deng, J. Tian and T. Liu, Synthesis and biological evaluation of salinomycin triazole analogues as anticancer agents, *Eur. J. Med. Chem.*, 2017, **127**, 900–908; (*c*) M. Sulik, E. Maj, J. Wietrzyk, A. Huczyński and M. Antoszczak, Synthesis and Anticancer Activity of Dimeric Polyether Ionophores, *Biomolecules*, 2020, **10**, 1039.

14 (*a*) S. Mronga, G. Mueller, J. Fischer and F. Riddell, A model for ion transport across membranes: solution structure of the ionophore metal complex salinomycin-Na determined by NMR and molecular dynamics calculations, *J. Am. Chem. Soc.*, 1993, **115**, 8414–8420; (*b*) E. F. Paulus, M. Kurz, H. Matter and L. Vértesy, Solid-state and solution structure of the salinomycin-sodium complex: Stabilization of different conformers for an ionophore in different environments, *J. Am. Chem. Soc.*, 1998, **120**, 8209–8221.

15 (*a*) Y. Miyazaki, H. Kinashi, N. Ōtake, M. Mitani and T. Yamanishi, Chemical modification and structure-activity correlation of salinomycin, *Agric. Biol. Chem.*, 1976, **40**, 1633–1640; (*b*) B. Borgström, X. Huang, M. Pošta, C. Hegardt, S. Oredsson and D. Strand, Synthetic modification of salinomycin: Selective O-acylation and biological evaluation, *Chem. Commun.*, 2013, **49**, 9944–9946.

16 A. Versini, L. Colombeau, A. Hienzsch, C. Gaillet, P. Retailleau, S. Debieu, S. Müller, T. Cañeque and R. Rodriguez, Salinomycin derivatives kill breast cancer stem cells by lysosomal iron targeting, *Chem. – Eur. J.*, 2020, **26**, 7416–7424.

17 W. Zhang, J. Wu, B. Li, J. Xia, H. Wu, L. Wang, J. Hao, Q. Zhou and S. Wu, Synthesis and biological activity evaluation of 20-epi-salinomycin and its 20-*O*-acyl derivatives, *RSC Adv.*, 2016, **6**, 41885–41890.

18 (*a*) Q. Shi, Y. Li, S. Bo, X. Li, P. Zhao, Q. Liu, Z. Yang, H. Cong, H. Deng, M. Chen, S. Chen, X. Zhou, H. Ding and Z. Jiang, Discovery of a (19)F MRI sensitive salinomycin derivative with high cytotoxicity towards cancer cells, *Chem. Commun.*, 2016, **52**, 5136–5139; (*b*) Y. Li, Q. Shi, J. Shao, Y. Yuan, Z. Yang, S. Chen, X. Zhou, S. Wen and Z. Jiang, Synthesis and biological evaluation of 20-epi-amino-20-deoxysalinomycin derivatives, *Eur. J. Med. Chem.*, 2018, **148**, 279–290.

19 (*a*) S. Emami, M. Falahati, A. Banifatemi, M. Amanlou and A. Shafiee, (E)-and (Z)-1, 2, 4-Triazolylchromanone oxime ethers as conformationally constrained antifungals, *Bioorg. Med. Chem.*, 2004, **12**, 3971–3976; (*b*) A. Karakurt, S. Dalkara, M. Ozalp, S. Ozbey, E. Kendi and J. P. Stables, Synthesis of some 1-(2-naphthyl)-2-(imidazole-1-yl) ethanone oxime and oxime ether derivatives and their anticonvulsant and antimicrobial activities, *Eur. J. Med. Chem.*, 2001, **36**, 421–433.

20 Y. Fan, J. Wu, X. Ke and Z. Huang, Design, synthesis and evaluation of oxime-functionalized nitrofuranylamides as novel antitubercular agents, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 3064–3066.

21 N. Matsumori, A. Morooka and M. Murata, Conformation and location of membrane-bound salinomycin-sodium complex deduced from NMR in isotropic bicelles, *J. Am. Chem. Soc.*, 2007, **129**, 14989–14995.

22 F. Wang, S. Zhou, D. Qi, S. Xiang, E. Wong, X. Wang, E. Fonkem, T. Hsieh, J. Yang, B. Kirmani, J. Shabb, J. Wu, M. Wu, J. Huang, W. Yu and E. Wu, Nucleolin is a functional binding protein for salinomycin in neuroblastoma stem cells, *J. Am. Chem. Soc.*, 2019, **141**(8), 3613–3622.