Salinomycin Hydroxamic Acids: Synthesis, Structure, and Biological Activity of Polyether Ionophore Hybrids

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S Supporting Information

ABSTRACT: The polyether ionophore salinomycin has recently gained attention due to its exceptional ability to selectively reduce the proportion of cancer stem cells within a number of cancer cell lines. Efficient single step strategies for the preparation of hydroxamic acid hybrids of this compound varying in N- and O-alkylation are presented. The parent hydroxamic acid, salinomycin-NHOH, forms both inclusion complexes and well-defined electroneutral complexes with potassium and sodium cations via 1,3-coordination by the hydroxamic acid moiety to the metal ion. A crystal structure of an cationic sodium complex with a noncoordinating anion corroborates this finding and, moreover, reveals a novel type of hydrogen bond network that stabilizes the head-to-tail conformation that encapsulates the cation analogously to the native structure. The hydroxamic acid derivatives display down to single digit micromolar activity against cancer cells but unlike salinomycin selective reduction of ALDH⁺ cells, a phenotype associated with cancer stem cells was not observed. Mechanistic implications are discussed.

KEYWORDS: salinomycin, ALDH⁺, head-to-tail conformation, hydroxamic acid derivative

A amalgamation of structural elements from two or more bioactive compounds into a hybrid structure is an attractive strategy in the design of functional analogues.^{1–3} In principle, such hybrids can expedite syntheses through simplified structures, provide mechanistic insight, and advantageously modulate properties like bioavailability, stability, and selectivity.^{4–18} A structure of interest in this context is the polyether ionophore salinomycin (SA, Figure 1).¹⁹ In addition to its industrial use as an anticoccidial and growth promoter, this compound and its more active semisynthetic analogues²⁰ have been shown to efficiently and selectively inhibit properties

associated with breast cancer stem cells (CSC).^{21–23} More recently, SA has also been shown to reduce scarring during wound healing.²⁴ Several studies have been directed at conversion of the carboxylate moiety of this compound and the related monensin into the corresponding esters and amides to impede the ability of electroneutral transport of metal ions across bilayers.^{25–31} Particularly secondary amides of monensin display an increased selectivity for relaxing sodium gradients in this context.³²

In light of this, we envisioned hydroxamic acid hybrid structures as attractive targets that would combine a novel 1,3-coordination motif capable of binding alkali metal ions like K⁺ and Na⁺ with a hydrophobic encapsulation by the SA polyether scaffold. In particular, such hybrids would be well suited as probes for investigating a connection between ionophore properties and selective effects against CSCs. Despite the considerable interest in hydroxamic acids in natural product hybrids,^{33–38} as siderophores,³⁹ inhibitors of stat3,⁴⁰ histone deacetylase (HDAC),^{41,42} and Ras signaling,⁴³ as well as isosters for carboxylic acids,⁴⁴ no such analogues have been described for polyether ionophores. A single study by Miller on the pore forming depsipeptide daptomycin highlights two hydroxamic acid derivatives of a natural product with antiporter activity.⁴⁵ Here, we present efficient pathways relying on direct

Figure 1. Polyether ionophores and hydroxamic acid natural products.

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Scheme 1. Systematic Synthesis of Hydroxamic Acid Hybrid Analogues of 5a–d^a

^a(a) Thermal ellipsoids are drawn at 30% probability. Selected hydrogen atoms involved in hydrogen bonding are shown. White = hydrogen atom; red = oxygen atom; blue = nitrogen atom; purple = sodium atom. (b) The PF_6^- counter ion and a solvate EtOAc are removed for clarity.

carboxylate activation to hydroxamic acid analogues of SA systematically varying in alkylation at the hydroxamate nitrogen and oxygen. Structural and functional investigations of the resulting library reveal down to single digit micromolar activity against JIMT-1 and MCF-7 breast cancer cells. In contrast to the native structure, however, little or no selectivity was found against aldehyde dehydrogenase positive cells (ALDH^+), a phenotype associated with CSC properties such as high tumorigenicity and increased migration.^{46,47}

Efficient synthesis of unsubstituted hydroxamic acids is challenging, in part due to the hydrolytic sensitivity of many such products.^{48,49} In light of this and of the low accessibility of the carboxylate moiety of SA,²⁰ it is noteworthy that single step access to each of the targeted hybrid derivatives 5a–d could be attained (Scheme 1). Especially, as a classical method like aminolysis with NH_2OH when applied to salinomycin methyl ester failed to give even trace amounts of product. Minor variations in the reaction conditions such as the choice of uronium-type coupling reagent minimized both side product formation and decomposition of the sensitive structures and enabled isolation of each analogue by flash chromatography.

The parent compound in the series, hydroxamic acid, 5a, was synthesized with complete selectivity for *N*-acylation by reacting SA with free base hydroxylamine in DMF using HATU as the coupling reagent. A two-step protocol via formation of an *O*-*tert*-butyldimethylsilyl (TBS) protected intermediate followed by removal of the silyl group with

TBAF was also evaluated but gave lower yields over the two steps. Despite extensive experimentation, we were unable to crystallize 5a or its Na^+ or K^+ salts. Drawing on a report of a neutral NaClO_4 complex of monensin, however,^{50,51} we were gratifyingly able to obtain single crystals of a neutral form of 5a binding a sodium atom with a noncoordinating PF_6^- counterion. On a general note, this result suggests that alkali metals with noncoordinating counterions may be of a broader utility for growing crystals of related compounds that are otherwise difficult to crystallize. The scXRD structure of 5a·NaPF₆ corroborated the structural assignment and, moreover, the premise that such derivatives are capable of neutral metal cation coordination via 1,3-binding of the cation by the hydroxamic acid moiety. Similarly to the crystal structure of SA·Na,⁵² 5a·NaPF₆ shows coordination to the metal ion from the oxygen atoms of the D- and E-rings as well as from the C11 carbonyl group. The acyl group in 5a·NaPF₆, however, displays a strikingly different behavior from that of the carboxylate of SA·Na. The head-to-tail conformation in SA·Na is stabilized by hydrogen bonds from the C9 and C28 hydroxyl groups to the carboxylate, which are absent in 5a·NaPF₆. Instead, a novel type of hydrogen bond network stabilizes a similar conformation wherein the C20 hydroxyl group serves as hydrogen bond donor⁵³ to the hydroxamate OH ($\text{O}\cdots\text{O} = 2.9 \text{ \AA}$) that in turn donates a hydrogen bond to the C28 oxygen atom ($\text{O}\cdots\text{O} = 2.6 \text{ \AA}$).

two concentrations (5 and 10 μM), no or only minor selectivity against this phenotype.

In line with this result, **5a** requires over 100-fold higher concentrations to show similar activity in relaxing alkali metal ion gradients across membranes of large unilamellar vesicles compared to SA (Figure 3, see Supporting Information for

Figure 3. H^+/K^+ exchange across a lipid membrane mediated by derivative **5a–d** and salinomycin. Ion exchange was monitored by the acidification inside large unilamellar vesicles (lipid ratio POPC/POPG 3:1) as determined by the pH reporter pyranin.

details). Our interpretation of the lack of selectivity against ALDH^+ cells, even at concentrations where cell proliferation is considerably impeded, is that the primary mechanism of toxicity exhibited by the hydroxamic acid hybrids is altered from that of SA.⁵⁷ These findings are in line with our previous studies where only salinomycin analogues with a free carboxylic acid give phenotype selectivity.²²

In conclusion, efficient single step methods for preparation of a library of hydroxamic acid hybrids of the polyether ionophore SA are presented. The products exemplify a novel type of hybrid structure between hydroxamic acids and polyether ionophores and include the first example of a C1-modified analogue of SA with a demonstrated ability to form both well-defined neutral complexes with alkali metal ions and inclusion complexes with NaCl and KCl. An scXRD structure of hydroxamic acid **5a** exhibits a novel type of hydrogen bond network that stabilizes a head-to-tail conformation, which encapsulates the bound metal ion. Metal ion exchange and transport experiments further show that such complexes can participate in metal ion translocation across biological membranes, although less efficiently than the native structure. The hydroxamic acid derivatives exhibit low micromolar activities against breast cancer cells, but unlike the native structure, little to no selectivity for reduction of the proportion of ALDH^+ cells, a phenotype associated with CSC properties, was found. Mechanistically, the results point to a different primary origin of the observed cytotoxicity effects by such derivatives compared to that of SA. Additionally the results underscore that the phenotype selectivity of salinomycin appears to originate from a capacity for efficient ion transport. Further studies on these and related structures against CSCs and as mechanistic probes are currently under way and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.6b00079.

Experimental procedures and characterization for compounds **5a**, **5b**, **5c**, **5d**, **6**, **5a**- NaPF_6 , **7**, and **8**. Structure elucidation of all compounds. Copies of ^1H and ^{13}C NMR spectra for all new compounds. Procedures for ion exchange and transport experiments. Experimental details of the MTT and ALDEFLUOR assays (PDF) scXRD data for **5a**, **5b**, and **5d** (CCDC Accession Codes: 1446108, 1446121, 1446122) (CIF)

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Notes

The authors declare no competing financial interest.

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