Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and biological evaluation of santacruzamate A analogues for anti-proliferative and immunomodulatory activity

Samantha M. Gromek [a], James A. deMayo [a], Andrew T. Maxwell [a], Ashley M. West [a], Christopher M. Pavlik [a], Ziyan Zhao [b], Jin Li [a], Andrew J. Wiemer [a], Adam Zweifach [b], Marcy J. Balunas [a,*]

[a] Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, 69 N. Eagleville Rd, Storrs, CT 06269, USA
[b] Department of Molecular and Cell Biology, University of Connecticut, 91 N. Eagleville Rd, Storrs, CT 06269, USA

ARTICLE INFO

Article history:
Received 27 June 2016
Revised 17 August 2016
Accepted 22 August 2016
Available online xxxx

Keywords:
Santacruzamate A
Natural product analogues
Enzyme assays
Anti-proliferative activity
Immune modulation

ABSTRACT

Santacruzamate A (SCA) is a natural product isolated from a Panamanian marine cyanobacterium, previously reported to have potent and selective histone deacetylase (HDAC) activity. To optimize the enzymatic and cellular activity, 40 SCA analogues were synthesized in a systematic exploration of the zinc-binding group (ZBG), cap terminus, and linker region. Two cap group analogues inhibited proliferation of MCF-7 breast cancer cells, with analogous increased degranulation of cytotoxic T cells (CTLs), while one cap group analogue reduced CTL degranulation, indicative of suppression of the immune response. Additional testing of these analogues resulted in reevaluation of the previously reported SCA mechanism of action. These analogues and the resulting structure–activity relationships will be of interest for future studies on cell proliferation and immune modulation.

1. Introduction

Development of new cancer chemotherapeutic compounds is a fundamentally challenging endeavor requiring considerations at the in vitro, in vivo, preclinical, and clinical stages. Ideal compounds for drug discovery and development target cancerous cells while maintaining low toxicity to normal cells, with an ongoing shift away from discovery of new compounds with broad cytotoxicity to those with selective activity, including compounds that specifically regulate gene expression via epigenetic modulation, that target signaling pathways, or that enhance immune system function.[1,2] Recently, epigenetic modulators such as inhibitors of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) have received increased attention as anti-cancer agents, although their clinical utility has thus far been limited to leukemias and lymphomas.[3,4] Likewise, there has been considerable attention towards the development of small molecule cancer therapeutics that affect immune system targets, although there are still very few currently available agents.[5–8]

HDACs and histone acetyltransferases (HATs) are key enzymes regulating the packaging of DNA around histones, having extensive impacts on gene transcription. HDAC enzymes remove acetyl groups from histone lysine residues, resulting in enhanced binding affinity with DNA and leading to repression of gene transcription. Regulation of acetylation and deacetylation patterns allows for acute control of differentiation, growth arrest, and apoptosis.[9–11] Moreover, hyper-deacetylation can result in silencing of tumor suppression genes, loss of apoptosis, and initiation and propagation of tumorogenesis.[12] Four HDAC inhibitors are currently approved by the United States Food and Drug Administration (US FDA) including suberoylanilide hydroxamic acid (SAHA, Vorinostat®, Fig. 1), romidepsin (Istodax®), panobinostat (Farydak®), and belinostat (Beleodaq®), all of which are approved for patients with refractory cancers (lymphoma or myeloma).[13] HDACs exist in several classes and with many isozymes, with current research focused on the discovery and development of isozyme selective epigenetic modulators to avoid the side effects inherent in non-selective inhibitors.[14–16]

Targeted therapies that induce anti-cancer immunity have been the focus of intensive research efforts, with recent efforts to discover new small molecule cancer immunotherapeutics.[8] Components of the adaptive and innate immune systems are capable of recognizing and killing cancer cells, initiated by tumor antigens that activate cytotoxic T lymphocytes (CTLs) through T cell receptors or by cell-surface molecules that activate natural killer cells.[17] Although there are several clinically available monoclonal antibodies that modulate immune function, imiquimod (Aldara®) is one of the only small molecule immunomodulators approved for cancer patients in the US and is limited to cutaneous malignancies.[7]

* Corresponding author. Tel.: +1 860 486 3051; fax: +1 860 486 6857.
E-mail address: marcy.balunas@uconn.edu (M.J. Balunas).

http://dx.doi.org/10.1016/j.bmc.2016.08.040
0968-0896/

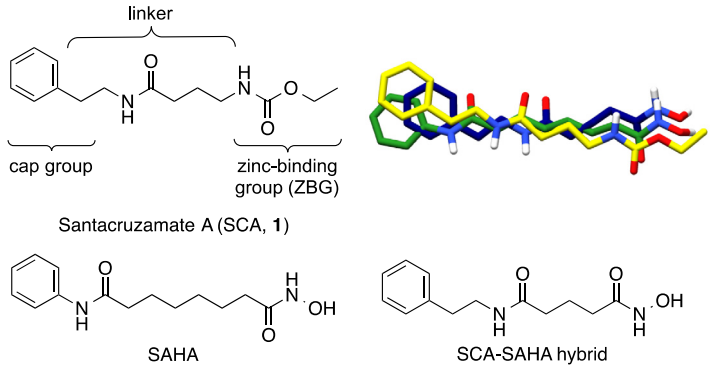


Figure 1. Santacruzamate A (SCA, **1**), SAHA, and the SCA-SAHA hybrid compound reported previously,[18] and their overlaid 3D structures (SCA in yellow, SAHA in green, and SCA-SAHA hybrid in blue).

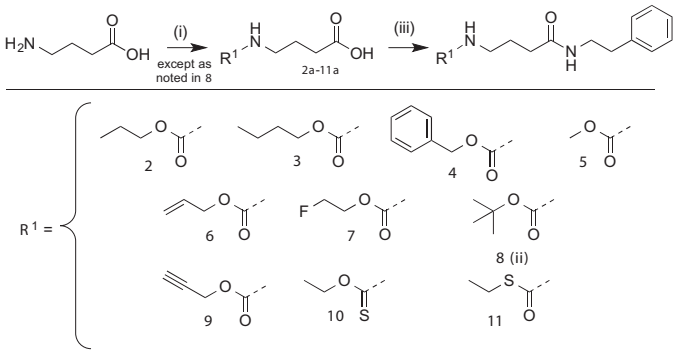
We initially identified santacruzamate A (SCA, **1**, Fig. 1) as a bioactive natural product isolated from the marine cyanobacterium, cf. *Symploca* sp.[18] SCA consists of three structural moieties including an ethyl carbamate terminus, a modified γ-aminobutyric acid (GABA) linker, and a phenethylamine cap group and has several structural features in common with SAHA (Fig. 1), which led to an initial investigation of SCA for HDAC inhibition. SCA, SAHA, and most HDAC inhibitors contain three basic structural motifs including a zinc-binding group (ZBG), an aliphatic linker, and a surface recognition cap group (Fig. 1). In contrast to SCA, SAHA ends in a hydroxamate terminus and has a longer 6-carbon linker directly attached to the phenylamine cap. SAHA binds to HDAC with the phenyl cap group resting on top of the enzyme pocket, the aliphatic linker traversing through the small channel, and the hydroxamate binding to the zinc at the bottom.[19] Because most hydroxamic acids suffer from pan-isozyme activity and several clinical liabilities (e.g., poor oral absorbance, rapid hydrolysis yielding poor pharmacokinetics, and strong non-specific affinity for metalloproteins),[20] we were enthusiastic about SCA given the carbamate terminus. With its relatively small, linear modified peptide structure, SCA is easily amenable to synthetic modifications and advanced biological evaluation to fully explore structure–activity relationships. In an effort to optimize the enzyme activity and to enhance the cellular activity, we performed a systematic exploration of the three chemical regions of the SCA structure including modification of the zinc-binding group (ZBG), cap terminus, and linker region, resulting in 40 derivatives reported herein that were evaluated for anti-proliferative and immunomodulatory activity.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of santacruzamate A zinc-binding group analogues

Given the known importance of ZBG binding for HDAC inhibition, the first structural modifications we made were to the carbamate terminus of SCA (Schemes 1–5). With SAHA, the hydroxamate allows for bi-dentate binding of zinc between the deprotonated terminal hydroxyl and the carbonyl oxygen with the catalytic pocket (see Fig. 3 and Section 2.3 for more details). This bi-dentate binding facilitates the strong metal binding characteristics of hydroxamate bearing compounds. Herein, SCA is hypothesized to exhibit monodentate coordination to the zinc and thus modification centered on adjusting the electronics around the carbonyl oxygen of the SCA carbamate. For this first phase we completed the synthesis of 20 compounds that differ in their ZBG terminus, leaving the three carbon aliphatic linker and phenethylamine cap group unaltered.



Scheme 1. Synthesis of santacruzamate A *N*-carbamate analogues. Reagent and conditions: (i) $ClCO_2$-R^1, K_2CO_3 or NaOH, THF, 0 °C to rt, 70–90%; (ii) Boc_2O, NaOH, THF, 97%; (iii) phenethylamine, EDC–HCl, TEA, cat. DMAP, CH_2Cl_2, 43–93%.

N-Carbamate modifications (Scheme 1) included changing the ethyl carbamate to a methyl (**5**), a larger *tert*-butyl (**8**), elongating to a propyl (**2**) and butyl (**3**), elongating and adding unsaturation (**6** and **9**), an even larger phenyl (**4**), fluorination (**7**), and incorporating a thiocarbamate (**10** and **11**). Carbamate intermediates (Scheme 1, **2a**–**5a**, **7a**–**11a**) were all prepared via analogous synthetic methodology involving coupling of GABA with the respective chloroformate.[21–23] Compound **8a** was formed using standard di-*tert*-butyl dicarbonate (Boc_2O) protection.[24] To prepare compound **10a**, prior to coupling with GABA, sodium ethoxide was added to thiophosgene and reacted to obtain the ethyl chlorothioformate **10a**. All resulting carbamates were coupled to phenethylamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), trimethylamine (TEA), and catalytic 4-dimethylaminopyridine (cat. DMAP) yielding analogues **2**–**11**.

Ten additional ZBG analogues were also prepared. To explore the possibility of incorporation of bi-dentate binding, compounds **13** and **14** were synthesized (Scheme 2). A simpler urea analogue was also prepared (**12**). In another series, the carbamate was exchanged for amide bonds with various termini (Scheme 3, **15**–**19**). In addition, due to the potent zinc chelating affinity of the terminal thioester of largazole,[25,26] we replaced the carbamate with an acetylated thiol (Scheme 4, **20**). Lastly, in an effort to investigate the spatial orientation of the ethyl carbamate in the binding pocket an inverted ethyl carbamate, **21**, was synthesized (Scheme 5).

The three urea analogues (Scheme 2, **12**–**14**) were synthesized via **12a** starting from GABA which was dimerized using CS_2 to form a thiourea.[27] This thiourea intermediate was either carried through directly or oxidized to the urea intermediate upon exposure to 30% H_2O_2 and KOH to form **12b**.[28] Intermediate **12b** was then converted through to the ethyl urea following literature procedure,[27]