Bioorganic & Medicinal Chemistry Letters 19 (2009) 6588–6590

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of novel histone deacetylase inhibitor derived from nuclear localization signal peptide

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ARTICLE INFO

Article history:
Received 31 July 2009
Revised 6 October 2009
Accepted 7 October 2009
Available online 13 October 2009

Keywords:
HDAC
NLS
Histone deacetylase inhibition

ABSTRACT

We describe herein the synthesis and characterization of a new class of histone deacetylase (HDAC) inhibitors derived from conjugation of a suberoylanilide hydroxamic acid-like aliphatic-hydroxamate pharmacophore to a nuclear localization signal peptide. We found that these conjugates inhibited the histone deacetylase activities of HDACs 1, 2, 6, and 8 in a manner similar to suberoylanilide hydroxamic acid (SAHA). Notably, compound **7b** showed a threefold improvement in HDAC 1/2 inhibition, a threefold increase in HDAC 6 selectivity and a twofold increase in HDAC 8 selectivity when compared to SAHA.

Histone deacetylase (HDAC) inhibition has been clinically validated as a therapeutic strategy for cancer treatment with the FDA approval of suberoylanilide hydroxamic acid (SAHA) for the treatment of cutaneous T-cell lymphoma.[1] HDAC inhibitors (HDACi) have shown the ability to block angiogenesis and cell cycling, initiate differentiation and apoptosis. HDACi presumably derived their biological activities through perturbation of chromatin remodeling and acetylation states of key non-histone proteins.[2–7] Most HDACi, including SAHA, non-selectively inhibit the deacetylase activity of class I/II HDAC enzymes.[8,9] This broad HDAC inhibition is associated with reduced potency and toxic side effects. Attempts aimed at identifying isoform selective HDACi have been modestly successful, resulting in very few HDACi that are only partially isoform selective.[10–12]

HDACs 1 and 2, the primary targets for the anticancer activity of HDACi, are exclusively localized in the nucleus.[13,14] Thus, the development of a strategy for nuclear delivery and localization of HDACi could be an alternative approach to isoform selective HDACi. Toward this end, we sought novel peptide–HDACi conjugates that are capable of crossing both the plasma and nuclear membrane.[15] Most of the nuclear membrane-penetrating peptides described in the literature are derived from viral sources. Common examples include the Simian virus nuclear localization peptides (NLS),[15–17] HIV 1 Tat-protein derived peptides,[18] and peptides derived from adenovirus fiber protein. NLS peptides are primarily utilized by viruses to cross the nuclear membrane, making them an ideal candidate for HDACi conjugation. Additionally, we reasoned that NLS, with lysine-enriched sequences, could also act as a substrate-mimetic to HDAC by mimicking the N-terminal tail lysine residues of the core histones. We report here the identification of NLS-peptide derived HDACi with anti-HDAC activity and HDAC isoform selectivities that rival or better that of SAHA.

Our design approach involved conjugation of a SAHA-like aliphatic-hydroxamate HDAC inhibition group directly to the NLS peptide through 1,2,3-triazole moiety.[11] This very simple initial design could facilitate a facile, high yielding synthesis of the proposed NLS–HDACi conjugates. Accordingly, we prepared compounds **7a–c** having two 1,2,3-triazole rings connecting a NLS-derived peptide to a HDAC surface recognition group and a hydroxamate zinc binding group to the surface recognition group through flexible methylene linkers whose lengths are optimized for HDAC inhibition.[11,12]

The designed conjugates **7a–c** were synthesized through a six-step synthetic route as shown in Scheme 1. Cu(I)-catalyzed reaction of 4-ethynylaniline with azido esters **1a–c** gave cycloadducts **2a–c** in excellent yields.[11,19] The diazotization of **2a–c** by treatment with sodium nitrite followed by exposure of the crude products to sodium azide led to the azido derivatives **3a–c** in good yields. However, a portion of azido derivatives **3** was hydrolyzed into carboxylic acid giving a mixture of both ester and carboxylic acid derivatives. To hydrolyze the rest of the ester, lithium hydroxide hydrate was added to the mixture giving a complete conversion to the azido carboxylic acid derivatives **4a–c** in excellent yields. The reaction of acid **4a–c** with *O*-trityl hydroxylamine gave the desired *O*-trityl azido-triazolylhydroxamates **5a–c** that were subsequently coupled to the alkyne-terminated protected NLS peptide **PCS-37689-PI**[16] to give cycloadducts **6a–c**. Exposure of

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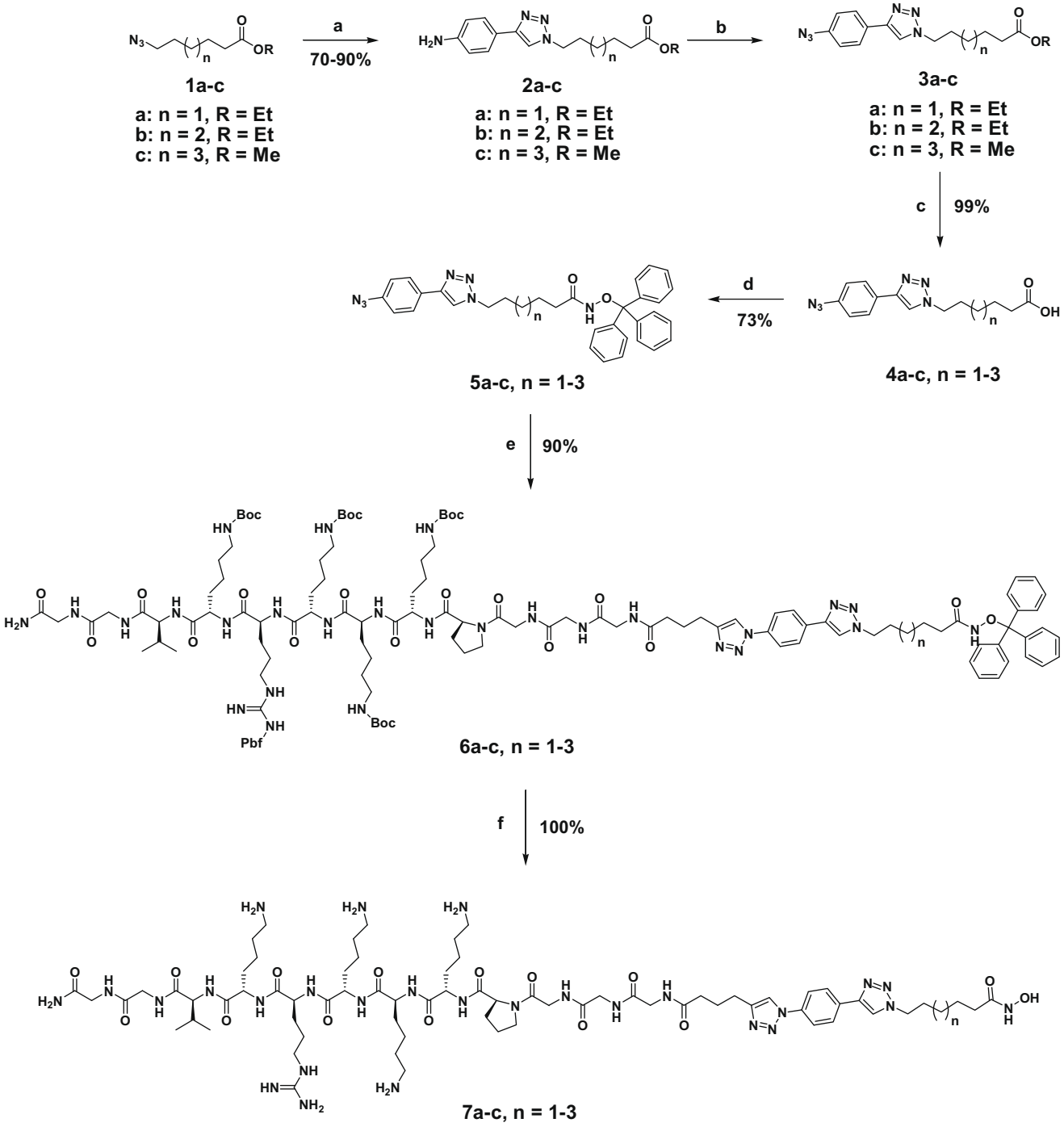
doi:10.1016/j.bmcl.2009.10.028



Scheme 1. Synthesis of NLS–HDACi conjugates. Reagents: (a) 4-ethynylaniline, CuI, DIPEA, THF; (b) $NaNO_2$, NaN_3, 17.2% $HCl_{(aq)}$; (c) $LiOH{\cdot}H_2O$, THF/H_2O, (d) *o*-tritylhydroxylamine, EDCI, HOBT, NMM, DMF; (e) alkyne-terminated NLS peptides, CuI, TBTA, DIPEA, THF/DMF; (f) 90:5:5 TFA/TIPS/phenol.

cycloadducts **6a–c** to TFA removed all protecting groups yielding the desired NLS–HDACi conjugates **7a–c** in near quantitative yields.

The HDAC inhibition activity of **7a–c** was tested using a cell free assay (*Fluor de Lys*).[20] We found that the NLS–HDACi conjugates display potent HDAC inhibition activities, similar to SAHA, that is somewhat linker-length dependent against HDAC 1 and 2 from HeLa cell nuclear extract (Table 1). An increase in the linker-length from C_6 to C_7 conferred a better anti-HDAC activity. The NLS–HDACi conjugates also presented similar isoform selectivity to that of SAHA with respect to HDAC 6 and 8 (Table 1). Compound **7b**, with a C_7 linker, stood out as it showed not only a threefold improvement in HDAC 1/2 activity when compared to SAHA, but also a twofold increase in HDAC 8 selectivity and a threefold increase in HDAC 6 selectivity.

Encouraged by the potent anti-HDAC activities of these NLS–HDACi conjugates, we then evaluated their whole cell anti-proliferative activities using trypan blue exclusion and MTT assay. Sadly, none of the conjugates exhibited any appreciable, anti-proliferative activity in DU-145 or HSC3 cell lines at drug concentrations up to 25 μM in both assays (data not shown). In an attempt to shed light on the lack of whole cell activity of these conjugates, we

Table 1
HDAC inhibitory activity and isoform selectivity of SAHA and NLS-linked compounds

	HDAC 1/2 IC50 (nM)	HDAC 8 IC_{50} (nM)	HDAC 8 selectivity[a]	HDAC 6 IC_{50} (nM)	HDAC 6 selectivity[a]
SAHA	49	2185	45	800	16
7a	36	3503	96	575	16
7b	14	1528	108	716	51
7c	25	1243	50	714	29

[a] Selectivity is the activity of the HDAC isoform (6 or 8) divided by the activity of HDAC 1/2.

prepared a BODIPY tagged analog of the NLS (Supplementary data).[21] The syntheses of alkyne BODIPY and NLS-BODIPY are described in Supplementary data (Schemes S1 and S2, respectively). The intracellular localization of the dye labeled NLS was then monitored using fluorescence microscopy at a variety of incubation times (Supplementary data). It was found that the dye labeled NLS was not taken up into the nucleus; instead it was sequestered in the cytosol. This preliminary result may provide an explanation for the poor whole cell activity of the compounds.

We have described a class of novel NLS-peptide derived HDACi. All of the compounds demonstrated HDAC inhibition and isoform selectivity similar to, and in the case of **7b** better than, SAHA. Despite these promising HDAC inhibitory activities, none of the compounds exhibited whole cell activity at concentrations up to 25 μM. The lack of appreciable whole cell activity of these NLS–HDACi conjugates may be partly due to their cytosolic entrapment. Efforts are currently underway in our laboratory to enhance the nuclear penetration of these conjugates by optimizing their NLS sequence linker, and HDAC inhibition moiety.

Acknowledgments

This work was financially supported by Georgia Institute of Technology, by the Blanchard fellowship and by NIH Grant R01CA131217. J.C.C. and P.C.C. are recipients of the GAANN predoctoral fellowship from the Georgia Tech Center for Drug Design, Development, and Delivery.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.028.

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