Tetrahedron Letters 54 (2013) 151–153

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Solid phase synthesis of hydroxamate peptides for histone deacetylase inhibition

David M. Wilson [a,*], Lisa N. Silverman [b], Markus Bergauer [b], Kayvan R. Keshari [a]

[a] Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, CA 94143, USA
[b] Department of Chemistry, Columbia University, New York, NY 10027, USA

ARTICLE INFO

Article history:
Received 26 September 2012
Revised 23 October 2012
Accepted 25 October 2012
Available online 2 November 2012

Keywords:
Histone deacetylase
Solid phase
Peptide
Hydroxamic acid
Cancer

ABSTRACT

An orthogonal protecting group strategy was devised to synthesize hydroxamic acid containing peptides for biomimetic histone deacetylase (HDAC) inhibition. The basic building block was a protected aminosuberic acid (Asu) derivative bearing a protected hydroxamate in the side-chain, related closely to HDAC inhibitors that are transition-state analogs of acetyllysine. These inhibitors include suberoylanilide hydroxamic acid (SAHA), currently being used to treat a variety of human cancers. This strategy was employed to synthesize a series of nonameric peptides related to actual HDAC substrates, derived from known sites of acetylation/deacetylation on the N-terminal tails of the histone core proteins H2A, H2B, H3, and H4. In each case the lysine residue was replaced by a hydroxamate-bearing side chain, to mimic the endogenous site of deacetylation. Mass spectrometry and high performance liquid chromatography (HPLC) confirmed the success of automated solid-phase synthesis. These results suggest facile synthesis of a new class of HDAC inhibitors that may have enhanced selectivity for specific HDAC isoforms.

The reversible acetylation of histones has been shown to play a critical role in transcriptional regulation.[1,2] This process is regulated by a number of nucleosome remodeling complexes and by post translational modifications to the core histones themselves,[3–5] a process regulated by the competing enzymatic activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs).[6] The targets of these enzymes are the N-terminal domains of all four core histone types, with an additional two C-terminal domains found on the C-termini of the histone 2As. It has been speculated that the specific patterns of post-translational events occurring at these sites comprise a code utilized in the recruitment of particular transcriptional complexes.[7]

Inhibitors of HDACs represent a promising modality for the treatment of human cancers, with several compounds in the clinic most notably suberoylanilide hydroxamic acid (SAHA), a zinc chelator.[8–10] HDAC inhibitors have shown to lead to cell cycle arrest, differentiation and/or apoptosis in tumor cells. Because of the zinc chelating properties of hydroxamic acids, considerable effort has been made to synthesize hydroxamic acid containing peptides as inhibitors of HDAC and other zinc metalloenzymes.[11–13] Efforts to improve the potency of SAHA-related inhibitors were facilitated by crystallographic studies of histone deacetylase-like protein (HDLP), a bacterial homolog of human HDAC1, with bound trichostatin A (TSA) or SAHA.[14] These studies revealed that chelation of zinc in the catalytic pocket is the primary mechanism of inhibition, and suggested a 'biomimetic' approach to inhibitor design, whereby the actual HDAC substrate (acetylated lysine) is used to construct related inhibitors.[15] This was accomplished via introduction of a chiral center using (L)-aminosuberic acid (Asu), leading to inhibitors that mimic the transition state for the hydrolysis of acetyllysine, as depicted in Figure 1.[16]

In this Letter we have extended this biomimetic approach to peptide-based inhibitors whose structures are derived from actual histone tail sequences, and report a novel methodology for incorporating hydroxamic acids in the amino acid side chains of peptides. This was accomplished using an orthogonal protecting group strategy, by synthesizing an Fmoc-protected building block. This compound may be used in standard solid phase peptide synthesis protocols, with liberation of the CO(NHOH) functionality under typical cleavage conditions.

The syntheses of Fmoc protected **3** were achieved as shown in Figure 2. In Method A we used the commercially available Fmoc-(L)-Asu-OMe (**1**). The ω-carboxylic acid was activated with oxalylchloride and reacted with para-methoxybenzyl protected hydroxylamine.[17] The ester **2** was cleaved using LiOH to give Fmoc-(L)-Asu(NHOPMB)-OH in 71% yield.[18] Given the high cost of **1** and the need for building block **3** in a gram scale range, an alternative route starting from enantiopure (L)-amino suberic acid (**4**) was considered. After Fmoc-protection under standard conditions, simultaneous protection of the amino group and the carboxylic acid was achieved by heating acid **5** with paraformaldehyde/TsOH under water removal to give the oxazolidin-5-one. A similar strategy with Cbz-protected derivatives was used before to enable the

* Corresponding author. Tel.: +1 415 353 1668; fax: +1 415 353 8593.
E-mail address: david.m.wilson@ucsf.edu (D.M. Wilson).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.10.113

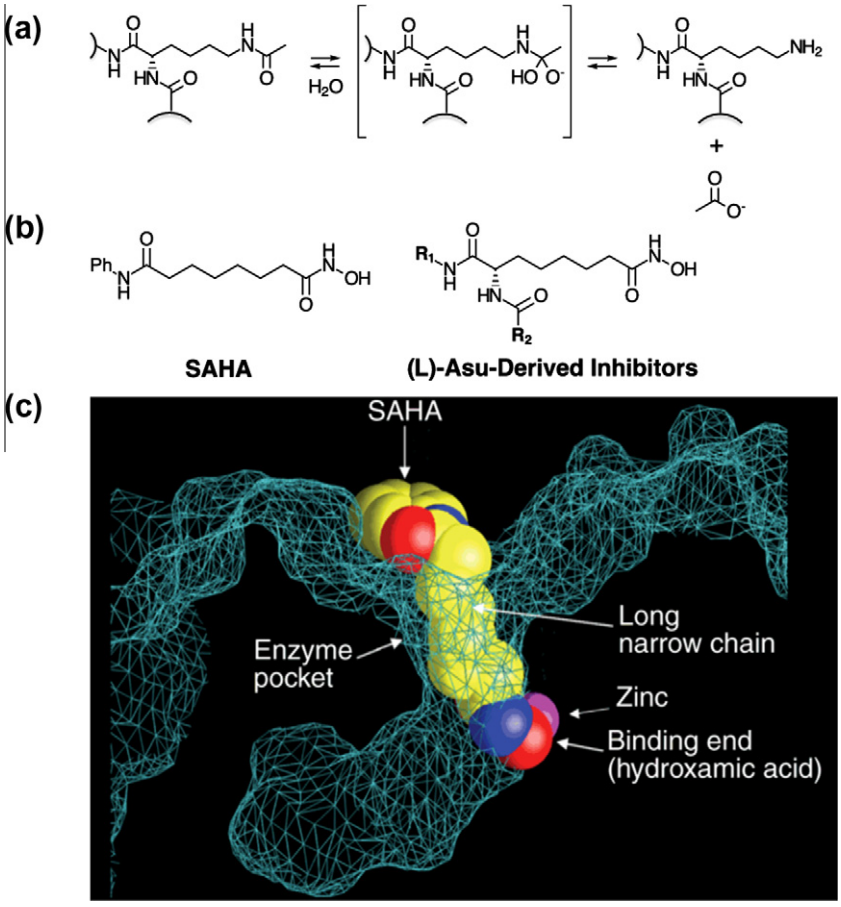

Figure 1. Molecular design of an aminosuberic acid-based inhibitor. (a) Action of HDACs on N-terminal acetyllysine residues, with zinc-mediated hydrolysis of side-chain acetyl group; (b) chemical structure of suberoylanilide hydroxamic acid (SAHA), a transition state analog of acetyllysine. Chiral (L)-Asu based hydroxamates mimic the endogenous substrate more closely and have been shown to be even more potent inhibitors of HDACs; (c) the X-ray crystallographic structure of histone deacetylase-like protein (HDLP) with bound SAHA was used to describe the Zn-mediated hydrolysis of acetyllysine residues at the N-terminus of histone core proteins, and has assisted in the design of HDAC inhibitors (adapted from Ref. 14).

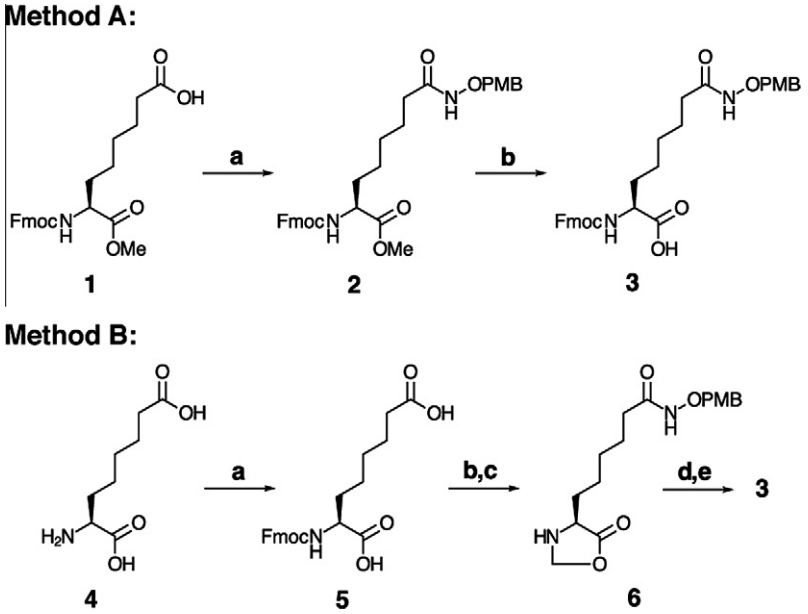

Figure 2. Method A: (a) (1) $(COCl)_2$ (1.1 equiv), CH_2Cl_2, cat. DMF, 0 °C to rt, 1 h; (2). H_2NOPMB (1.2 equiv), DIEA (2.0 equiv), CH_2Cl_2, 0 °C to rt., 2 h (88%); (b) $LiOH^*H_2O$ (0.2 M in H_2O, 2.0 equiv), THF, 0 °C, 8 h (71%). Method B: (a) FmocOSu (0.8 equiv), $NaHCO_3$ (3.0 equiv), 1,4-dioxane:H_2O (1:1), rt., 20 h (quant.); (b) paraformaldehyde (2.0 equiv), $TsOH^*H_2O$ (0.06 equiv), toluene, dean-stark, reflux, 3 h (93%); (c) (1) $(COCl)_2$ (1.2 equiv), CH_2Cl_2, cat. DMF, 0 °C to rt, 1 h; (2) H_2NOPMB (1.1 equiv), NEt_3 (1.3 equiv), CH_2Cl_2, 0 °C to rt., 2 h (77%); (d) THF, 1 N NaOH (1:1), rt., 3 h; (e) FmocOSu (1.5 equiv), $NaHCO_3$ (10.0 equiv), acetone:H_2O (1:1), rt., overnight (60%).

selective functionalization of amino acid side chains.[19,20] However, to the best of our knowledge, this is the first time that this strategy was applied to an Fmoc-protected amino acid. Activation of the free ω-carboxylic acid and reaction with H_2NOPMB, as in Method A, afforded compound **3**. Unfortunately treatment of the oxazolidin-5-one **6** with LiOH or NaOH did not give the free Fmoc-protected acid **3**, as reported previously for Cbz-protected analogous compounds.[19] Therefore the amino suberic acid derivative **6** was fully deprotected and subsequently reacted with FmocOSu under basic conditions to give the final product **3**. HPLC-analysis of Fmoc-(L)-Asu(NHOPMB)-OH using a chiral stationary phase[21] showed no racemization in the material obtained via Method A (99.9% ee). With Method B slight racemization was observed (93.8% ee).

The amino suberic acid derivative (L)-**3** was then used to synthesize a series of peptides corresponding to the N-termini of the histone core proteins, including all-(L)-Ac-PAPK-Asu(NHOH)-GSKK-$CONH_2$ in a continuous flow synthesizer on PAL-PEG-PS resin.[22] Figure 3 shows a crude HPLC chromatogram of the resulting hydroxamic acid-bearing peptide. The identities of all peptides were confirmed by mass spectroscopy. Preliminary studies indicate that these peptides are indeed potent inhibitors of human HDACs in vitro, results will be reported separately.

In conclusion, we have developed a novel orthogonally protected unnatural aminosuberic acid derivative, compatible with routine solid phase methods. This building block was used to synthesize a series of hydroxamic acid bearing peptides that mimic actual HDAC substrates, namely the acetylated N-termini of histone core proteins. This strategy may be employed to generate libraries of lead compounds that may have enhanced selectivity for specific HDAC isoforms.

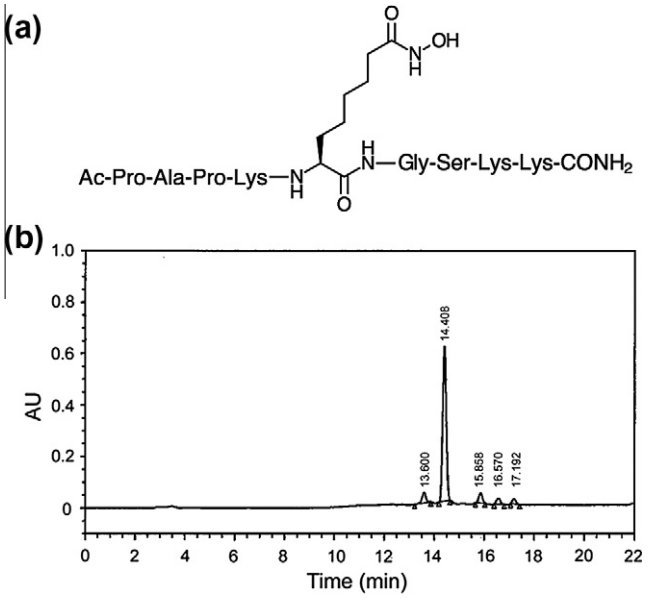

Figure 3. Synthesis of biomimetic peptide-based HDAC inhibitors. (a) Example of hydroxamate-bearing peptide constructed using building block **3** and automated solid-phase synthesis. The site of the artificial Asu-based amino acid corresponds to a known site of acetylation/ deacetylation on the N-terminus of histone core protein H2B; (b) Crude chromatogram, obtained by reversed phase C_{18}-HPLC using a water(+0.1% TFA)-acetonitrile (+0.1% TFA) gradient. Preparative HPLC with collection of the dominant peak and subsequent mass spectrometry confirmed the identity of all hydroxamate-bearing peptides.

Acknowledgements

The authors would like to acknowledge the support of Professor Ronald Breslow.

References and notes

1. Grunstein, M. *Nature* **1997**, *389*, 349–352.
2. Kouzarides, T. *Cell* **2007**, *131*, 822.
3. Zhang, Y.; Ng, H. H.; Erdjument-Bromage, H.; Tempst, P.; Bird, A.; Reinberg, D. *Genes Dev.* **1999**, *13*, 1924–1935.
4. Alland, L.; David, G.; Shen-Li, H.; Potes, J.; Muhle, R.; Lee, H. C.; Hou, H., Jr.; Chen, K.; DePinho, R. A. *Mol. Cell. Biol.* **2002**, *22*, 2743–2750.
5. You, A.; Tong, J. K.; Grozinger, C. M.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1454–1458.
6. Struhl, K. *Genes Dev.* **1998**, *12*, 599–606.
7. Strahl, B. D.; Allis, C. D. *Nature* **2000**, *403*, 41–45.
8. Duvic, M.; Talpur, R.; Ni, X.; Zhang, C.; Hazarika, P.; Kelly, C.; Chiao, J. H.; Reilly, J. F.; Ricker, J. L.; Richon, V. M.; Frankel, S. R. *Blood* **2007**, *109*, 31–39.
9. Kirschbaum, M.; Frankel, P.; Popplewell, L.; Zain, J.; Delioukina, M.; Pullarkat, V.; Matsuoka, D.; Pulone, B.; Rotter, A. J.; Espinoza-Delgado, I.; Nademanee, A.; Forman, S. J.; Gandara, D.; Newman, E. *J. Clin. Oncol.* **2011**, *29*, 1198–1203.
10. Marks, P. A. *Expert Opin. Investig. Drugs* **2010**, *19*, 1049–1066.
11. Dankwardt, S. M.; Billedeau, R. J.; Lawley, L. K.; Abbot, S. C.; Martin, R. L.; Chan, C. S.; Van Wart, H. E.; Walker, K. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2513–2516.
12. Komatsu, Y.; Tomizaki, K. Y.; Tsukamoto, M.; Kato, T.; Nishino, N.; Sato, S.; Yamori, T.; Tsuruo, T.; Furumai, R.; Yoshida, M.; Horinouchi, S.; Hayashi, H. *Cancer Res.* **2001**, *61*, 4459–4466.
13. Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 87–92.
14. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188–193.
15. Bertrand, P. *Eur. J. Med. Chem.* **2010**, *45*, 2095–2116.
16. Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097–5116.
17. Ramsay, S. L.; Freeman, C.; Grace, P. B.; Redmond, J. W.; MacLeod, J. K. *Carbohydr. Res.* **2001**, *333*, 59–71.
18. *(L)-2-N-Fmoc-amino-7-(4-methoxy-benzyloxycarbamoyl)-heptanoic acid ((L)-3)*: $C_{31}H_{34}N_2O_7$ (546.63): Mass (APCI+): 547 (M+1); HRMS (FAB+) calcd for $C_{31}H_{35}N_2O_7$: 547.2444, found: 547.2415; ^{1}H NMR (DMSO-d_6, 400 MHz): 1.1–1.7 (m, 8H), 1.93 (t, *J* = 7.2 Hz, 2H), 3.74 (s, 3H), 3.8–4.0 (m, 1H), 4.1–4.4 (m, 3H), 4.69 (s, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.32 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.41 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.88 (d, *J* = 7.5 Hz, 2H), 10.84 (s, 1H), 12.52 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): 24.77, 25.24, 27.99, 30.60, 32.17, 46.63, 53.72, 55.02, 65.53, 76.34, 113.61, 120.07, 125.24, 127.02, 127.59, 128.00, 130.52, 140.68, 143.82, 156.12, 159.27, 169.18, 173.96.
19. Scholtz, J. M.; Bartlett, P. A. *Synthesis-Stuttgart* **1989**, 542–544.
20. Itoh, M. *Chem. Pharm. Bull.* **1969**, *17*, 1679–1686.
21. (*S,S*)-Welk-O1 (25 cm × 46 mm, Regis Technologies); 50% isopropanol, 50% hexanes (+0.1% TFA), 1 ml/min, UV (254 nm): R_f 11.2 min ((L)-3) and 12.7 min ((D)-3). A racemic sample was obtained using Method B with rac-4.
22. *Peptide Synthesis*: Fmoc-protected amino acids were acquired as free acids from Bachem. The peptides were synthesized using the Fmoc strategy on a continuous flow Applied Biosystems Pioneer solid phase synthesizer using PAL-PEG-PS resin (0.2 mmol/g scale, continuous flow, Applied Biosystems). Single coupling cycles of 15 min using OH/HBTU activation chemistry (10-fold excess amino acid:resin) were employed for all residues except Asu, whereas OH/DIC/HOBt chemistry (four-fold excess amino acid:resin) and a 1 h coupling time were employed for the aminosuberate building block **3**. The side chain protecting groups used were as follows: Lys (*t*-Boc); Ser (O-*t*-Bu). After peptide assembly the N-terminus was manually acetylated using 1:1 acetic anhydride:pyridine for 30 min followed by thorough washing with DMF, methanol, and CH_2Cl_2. The peptide was cleaved from the resin and simultaneously deprotected using 95:2.5:2.5 (v/v/v) trifluoroacetic acid:triisopropyl silane:water for 3 h. The crude peptide was precipitated and washed with cold ether, followed by dissolution in water (0.1% v/v TFA), and lyophilization. Reversed phase C_{18} HPLC purification was used to purify the peptide to homogeneity using aqueous-acetonitrile gradients (0–100% acetonitrile, R_f: 14.4 min) containing 0.1% (v/v) TFA. After lyophilization, the identity of the peptide was confirmed by mass spectrometry (TOF, ES+: 1039 (M+1)).