Bioorganic & Medicinal Chemistry Letters 23 (2013) 3346–3348

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A cyclodextrin-capped histone deacetylase inhibitor

Jahangir Amin [a,*,†], Antonino Puglisi [a,b,*,†], James Clarke [b], John Milton [b], Minghua Wang [c], Ronald M. Paranal [d], James E. Bradner [d], John Spencer [a]

[a] Dept. of Chemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, East Sussex, UK
[b] Oxford Nanopore Technology Ltd Edmund Cartwright House, 4 Robert Robinson Avenue, Oxford Science Park, Oxford OX4 4GA, UK
[c] Terrence Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada M5S 3E1
[d] Dana–Farber Cancer Institute, 44 Binney Street, Dana Building, D510D, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 24 February 2013
Revised 18 March 2013
Accepted 22 March 2013
Available online 2 April 2013

Keywords:
HDAC Inhibitors
Cyclodextrin conjugates
Molecular modelling
Cancer

ABSTRACT

We have synthesized a β-cyclodextrin (βCD)-capped histone deacetylase (HDAC) inhibitor **3** containing an alkyl linker and a zinc-binding hydroxamic acid motif. Biological evaluation (HDAC inhibition studies) of **3** enabled us to establish the effect of replacing an aryl cap (in SAHA (vorinostat,)) **1** by a large saccharidic scaffold "cap". HDAC inhibition was observed for **3**, to a lesser extent than SAHA, and rationalized by molecular docking into the active site of HDAC8. However, compound **3** displayed no cellular activity.

Histone deacetylases are a family of metalloenzymes involved in chromatin remodeling. They play an important role in the epigenetic regulation of gene expression by removing acetyl groups from lysine residues in histones, which is associated with carcinogenesis and tumor progression.[1]

Histone deacetylase inhibitors (HDACis),[2] promote hyperacetylation of core histones leading to a relaxed chromatin, and are promising anticancer agents in cancer therapy.[3] SAHA **1** (suberoylanilide hydroxamic acid, vorinostat), for example, has been clinically approved for treating advanced cutaneous T-cell lymphoma (CTCL).[4]

The design of HDACis is often based on the molecular architecture of the HDAC acetyl lysine substrate, requiring (i) a metal-binding group allowing coordination to zinc, (ii) a linker that is of an appropriate chain length similar to that present in the acetyl–lysine substrate and (iii) a capping moiety (Fig. 1).

Furthermore, the rationale has been supported by a crystal structure of **1** in HDAC8 showing the deprotonated hydroxamic acid (metal ion chelator) binding to zinc, the alkyl linker occupying the hydrophobic channel and the anilide moiety interacting with the amino acid side groups at the entrance of the pocket in the active site.[5] The latter interaction engenders a possibility for engineering isoform selectivity[6] by modification of the capping

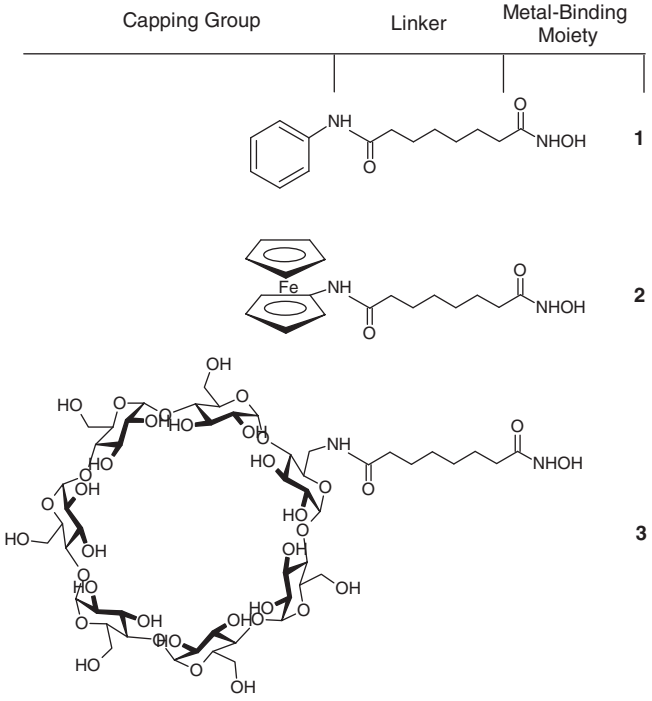


Figure 1. Examples of large aryl-cap containing HDACis.

* Corresponding authors. Fax: +44 1273 876687.
E-mail addresses: j.amin@sussex.ac.uk (J. Amin), antonino.puglisi@nanopore-tech.com (A. Puglisi).
† These authors contributed equally to this study.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.03.084

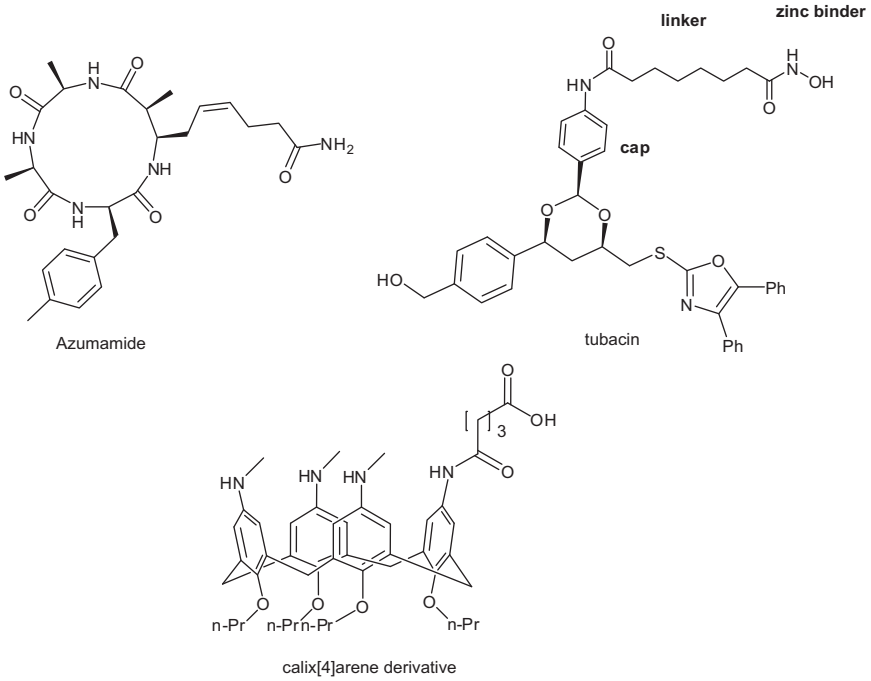


Figure 2. Examples of large aryl-cap containing HDACis.

group; for example, we have investigated transition metal containing HDACis, termed JAHAs **2**,[7] where a ferrocene group was employed as an aryl cap bioisostere.

Other researchers have looked at incorporating supramolecular architectures such as calix[4]arene caps,[8] with varying degrees of success in terms of selectivity and many natural or natural-product inspired products, including tubacin[9] and azumamides,[10] exhibit good HDAC isoform selectivity (Fig. 2).[11]

We describe, hereafter, preliminary results on the use of a cyclodextrin cap and its effect as a HDACi.

Cyclodextrins[12] (CDs) are a family of oligosaccharides composed of glucose units. These naturally occurring molecules take the tri-dimensional shape of a truncated cone with a hydrophilic exterior surface and an internal hydrophobic cavity. The three best characterised CDs are named α, β and γ with six, seven and eight units linked by α-1,4 glycosidic bonds, respectively. The covalent linkage of bioactive molecules to CDs has been proposed as an effective approach to (i) increase water solubility, (ii) improve the pharmacokinetics of the drug by preventing metabolism and excretion.[13]

Herein, we describe the synthesis and biological data concerning the effect of replacing the aryl capping group of SAHA with a β-cyclodextrin, as in **3** (Scheme 1) in an effort to explore the anticancer properties and HDAC inhibition of the resulting compound.

The synthesis of the CD-SAHA conjugate **3** is shown in Scheme 1. Treatment of the 6-deoxy-6-amino-β-cyclodextrin[14] **3a**, with methyl 8-chloro-8-oxooctanoate, under basic conditions, was followed by reaction of the resulting terminal ester with hydroxylamine, to afford the desired product **3**. The latter was characterised by NMR spectroscopy and mass spectrometry.

To explore the binding mode of **3** in deacetylases, docking studies were performed, using the structure of HDAC8–SAHA complex as a starting point (Fig. 3, based on PDB code:It69). This suggests that **3** is able to bind in a similar mode to SAHA **1** by maintaining the archetypal interactions that exist between the hydroxamate group and the zinc in the HDAC. Obviously, the cyclodextrin moiety in **3** occupies a larger space than the aryl cap in SAHA (Fig. 3). There are hydrogen bonding possibilities for residues on top of the cyclodextrin, however, the energy gain is anticipated to be compensated by the steric clashes engendered by the large cyclodextrin group. The binding mode suggested HDAC inhibition by **3** was feasible, although to a lesser extent than that of SAHA **1** (albeit, the MW of **3** is significantly higher compared to SAHA).

Table 1 summarizes the in vitro HDAC screening of **3** versus SAHA. Other HDAC isoforms (e.g., HDACs 4 and 5) were not inhibited. In general, we can see that **3** is roughly 5–10 times less potent than its simpler SAHA analogue yet it still shows IC50 values in the subnanomolar range, namely versus HDAC1 and HDAC6, affirming the docking studies. Cell-based studies were next performed; no

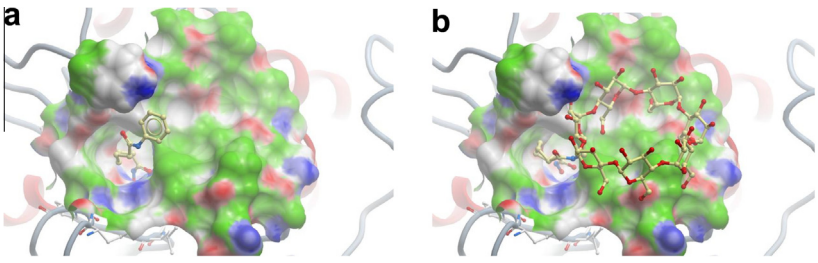
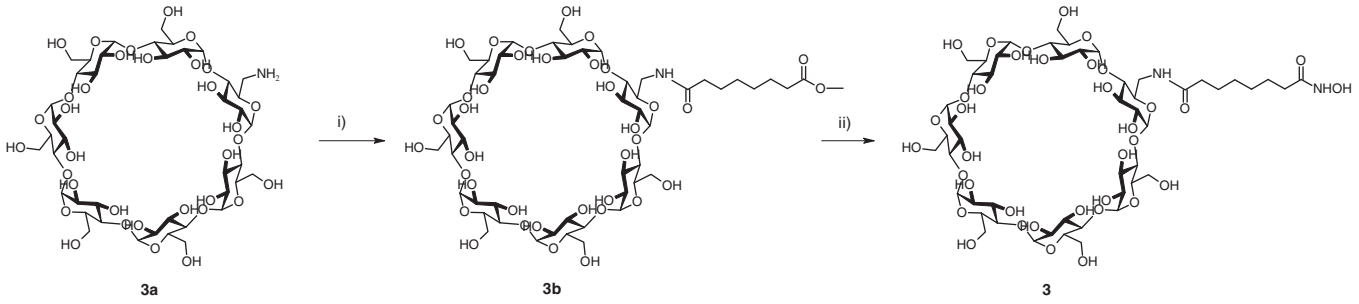


Figure 3. Docking poses of (a) SAHA **1** and (b) compound **3** in HDAC8.



Scheme 1. Synthesis of **3**. Reagents and conditions: (i) $ClCO(CH_2)_6CO_2CH_3$, rt, 24 h, pyridine; (ii) NH_2OH, KOH, 24 h, MeOH, under N_2.

Table 1
IC_{50} values for HDACis versus SAHA **1**[a]

Compound IC_{50} (μM) (SD)	HDAC1 IC_{50} (μM) (SD)	HDAC2 IC_{50} (μM) (SD)	HDAC3 IC_{50} (μM) (SD)	HDAC6 IC_{50} (μM) (SD)	HDAC8 IC_{50} (μM) (SD)
1	0.052 (0.003)	0.09 (0.01)	0.067 (0.006)	0.019 (0.002)	1.35 (0.009)
3	0.63 (0.05)	1.2 (0.1)	1.6 (0.2)	0.12 (0.01)	6 (0.3)

[a] IC_{50} values for compounds (μM) with standard deviation (SD) in parentheses. Assays were performed in duplicate.

effects on the leukaemia cancer cell line (K562) were, however, observed for **3**, pointing to a lack of cellular penetration for this analogue.

In conclusion, we have synthesized a HDACi bearing a bulky β-CD "cap" with moderate inhibition of HDACs, notably HDAC6, with an IC_{50} of 120 nM. We are currently aiming to perform X-ray studies on compound **3**. Moreover, we also intend to investigate smaller supramolecular structures such as more liphophilic α-cyclodextrins incorporated into HDACis combined with lipophilic chains given that similar three-dimensional cap groups were recently shown to exhibit excellent HDACi potency.[15]

Acknowledgments

Oxford Nanopore Technology Ltd is thanked for funding. The EPSRC Mass Spectrometry Service (Swansea University) is thanked for HRMS measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.084.

References and notes

1. Best, J. D.; Carey, N. *Drug Discovery Today* **2010**, *15*, 65.
2. Duvic, V.; Vu, J. *Expert Opin. Investig. Drugs* **2007**, *16*, 1111.
3. Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. *J. Med. Chem.* **2008**, *51*, 1505.
4. Marks, P. A. *Expert Opin. Investig. Drugs* **2010**, *19*, 1049.
5. Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B. C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. *Structure* **2004**, *12*, 1325.
6. Selected references: (a) Kozikowski, A. P.; Tapadar, S.; Luchini, D. N.; Kim, K. H.; Billadeau, D. D. *J. Med. Chem.* **2008**, *51*, 4370; (b) Li, G.; Jiang, H.; Chang, M.; Xie, H.; Hu, L. *J. Neurosci.* **2011**, *304*, 1; (c) Marek, L.; Hamacher, A.; Hansen, F. K.; Kuna, K.; Gohlke, H.; Kassack, M. U.; Kurz, T. *J. Med. Chem.* **2013**, *56*, 427; (d) Balasubramanian, S.; Verner, E.; Buggy, J. J. *Cancer Lett.* **2009**, *280*, 211; (e) Noureen, N.; Rashid, H.; Kalsoom, S. *Cancer Chemother. Pharmacol.* **2010**, *66*, 625.
7. (a) Spencer, J.; Amin, J.; Wang, M.; Packham, G.; Syed Alwi, S. S.; Tizzard, G. J.; Coles, S. J.; Paranal, R. M.; Bradner, J. E.; Heightman, T. D. *ACS Med. Chem. Lett.* **2011**, *2*, 358; (b) Spencer, J.; Amin, J.; Boddiboyena, R.; Packham, G.; Cavell, B. E.; Syed Alwi, S. S.; Paranal, R. M.; Heightman, T. D.; Wang, M.; Marsden, B.; Coxhead, P.; Guille, M.; Tizzard, G. J.; Coles, S. J.; Bradner, J. E. *Med. Chem. Commun.* **2012**, *3*, 61; (c) Librizzi, M.; Longo, A.; Chiarelli, R.; Amin, J.; Spencer, J.; Luparello, C. *Chem. Res. Toxicol.* **2012**, *25*, 2608.
8. Chini, M. G.; Terracciano, S.; Riccio, R.; Bifulco, G.; Ciao, R.; Gaeta, C.; Troisi, F.; Neri, P. *Org. Lett.* **2010**, *23*, 5382.
9. Tang, W.; Luo, T.; Greenberg, E. F.; Bradner, J. E.; Schreiber, S. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2601. and references cited therein.
10. Nakao, Y.; Yoshida, S.; Matsunaga, S.; Shindoh, N.; Terada, Y.; Nagai, K.; Yamashita, J. K.; Ganesan, A.; van Soest, R. W. M.; Fusetani, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 7553.
11. For a comprehensive review on HDACi isoform selectivity, see Bieliauskas, A. V.; Pflum, M. K. H. *Chem. Soc. Rev.* **2008**, *37*, 1402.
12. (a) Szejtly, J. *Chem. Rev.* **1998**, *98*, 1743; (b) D'Souza, V. T.; Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1741.
13. (a) Minami, K.; Hirayama, F.; Uekama, K. *J. Pharm. Sci.* **1998**, *87*, 715; (b) Uekama, K.; Minami, K.; Hirayama, F. *J. Med. Chem.* **1997**, *40*, 2755.
14. Puglisi, A.; Spencer, J.; Clarke, J.; Milton, J. *J. Inclusion Phenom. Macroycl. Chem.* **2012**, *73*, 475.
15. Islam, N. M.; Kato, T.; Nishino, N.; Kim, H.; Ito, A.; Yoshida, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 997.