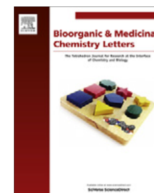
Bioorganic & Medicinal Chemistry Letters 22 (2012) 5025–5030

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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



Design, synthesis, modeling, biological evaluation and photoaffinity labeling studies of novel series of photoreactive benzamide probes for histone deacetylase 2

Aditya Sudheer Vaidya [a,†], Bhargava Karumudi [a,†], Emma Mendonca [b], Antonett Madriaga [a], Hazem Abdelkarim [a], Richard B. van Breemen [a], Pavel A. Petukhov [a,*]

[a] Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

[b] Department of Bioengineering, University of Illinois at Chicago, 851 South Morgan Street, Chicago, IL 60607, USA

ARTICLE INFO

Article history:
Received 12 April 2012
Revised 4 June 2012
Accepted 6 June 2012
Available online 18 June 2012

Keywords:
Benzamides
HDAC 1 and 2
Time-dependent inhibition
Photoaffinity labeling
Diazides

ABSTRACT

The design, modeling, synthesis, biological evaluation of a novel series of photoreactive benzamide probes for class I HDAC isoforms is reported. The probes are potent and selective for HDAC1 and 2 and are efficient in crosslinking to HDAC2 as demonstrated by photolabeling experiments. The probes exhibit a time-dependent inhibition of class I HDACs. The inhibitory activities of the probes were influenced by the positioning of the aryl and alkyl azido groups necessary for photocrosslinking and attachment of the biotin tag. The probes inhibited the deacetylation of H4 in MDA-MB-231 cell line, indicating that they are cell permeable and target the nuclear HDACs.

© 2012 Elsevier Ltd. All rights reserved.

Histone deacetylases (HDACs) are considered viable drug targets for multiple therapeutic applications including cancer and neurological diseases.[1,2] Recently, Cravatt et al.[3] and Gottesfeld et al.[4] described the design and applications of photoaffinity probes for profiling HDACs in native proteomes and live cells. The scaffold of the probes included a portion of a pan HDAC inhibitor suberoyl anilide hydroxamic acid (SAHA), a benzophenone group as a photoreactive group, and an alkyne handle to attach an azide containing reporter tag via (3+2) cycloaddition. Attempts to use the same features based on HDAC1 and 2 selective benzamide scaffolds resulted in probes with HDAC potency above 180 μM in HeLa cell nuclear lysate.[5]

We have already established the Binding (E) nsemble (Pro) filing with (F) photoaffinity (L) abeling approach (BEProFL) where we have experimentally mapped the multiple binding modes of diazide based photoreactive probes for HDACs.[6] The design of these probes included decoration of HDAC ligands with a 3-azido-5-azidomethylene moiety, a photoaffinity labeling group originally proposed by Suzuki et al.[7] for specific labeling of the catalytic portion of HMG-CoA reductase. The aromatic azido moiety was used as a photoreactive group and the aliphatic azide was well suited for (3+2) cycloaddition with an alkyne moiety of the biotin-containing reporter group. Based on these features, we have successfully designed and synthesized highly potent and selective probes for HDAC3 and HDAC8 and demonstrated that they are cell permeable and exhibit excellent antiproliferative activity against several cancer cell lines.[8] Our main objective in this study was to design photoreactive benzamide probes for HDAC2 and evaluate their activity/selectivity profile for other class I HDAC isoforms.

We hypothesized that a set of potent and selective benzamide-based probes capable of crosslinking with HDAC2 can be designed by appropriately decorating benzamides **1** and **2** (Fig. 1) with a combination of the aryl and alkyl azides. Both **1** and **2** and their derivatives were reported by Delorme,[9] Miller,[10] Gangloff,[11] and their colleagues to be active and selective inhibitors of HDACs1 and 2.

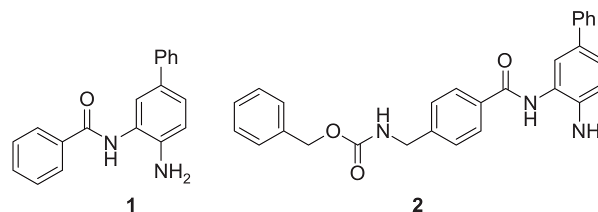


Figure 1. Benzamide inhibitors of HDAC 1 and 2.

* Corresponding author. Tel.: +1 312 996 4174; fax: +1 312 996 7107.
E-mail address: pap4@uic.edu (P.A. Petukhov).

† These authors contributed equally to the work.

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.017

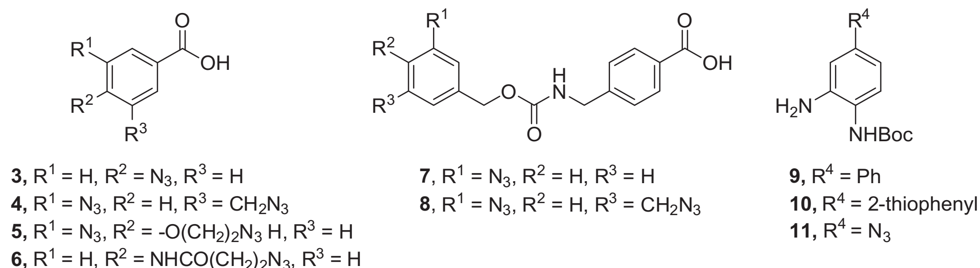


Figure 2. Amine and acid precursors used for synthesis of photoreactive probes.

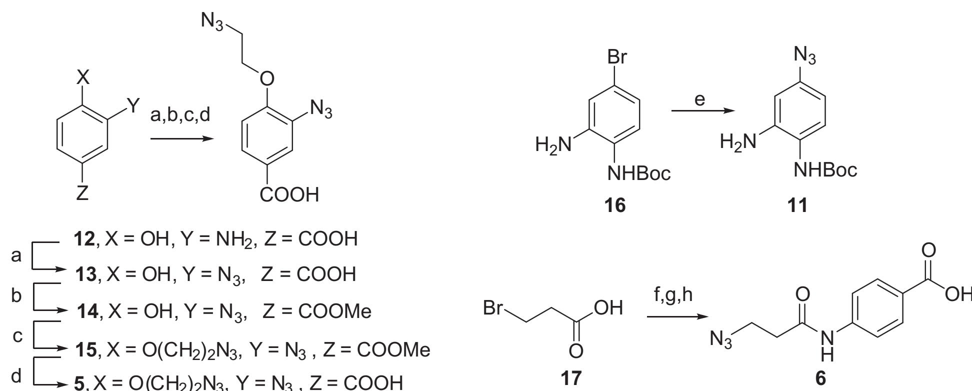
Substituted benzoic acids **3**–**8**, mono-*N*-Boc protected phenylenediamines **9**, **10** and azidoaniline **11** shown in (Fig. 2) were chosen as precursors for the synthesis of the photoreactive probes. Acid **4**, protected phenylendiamines **9**, **10**, and intermediates **16**, **18** and **19** were synthesized as reported previously,[7,8,10,12] whereas benzoic acid **3** was available commercially. The synthesis of precursors **5**, **6**, **7**, **8**, and **11** is shown in Schemes 1 and 2. The synthesis of the probes **1a**–**g** and **2a**–**b** proceeded through an efficient carbodiimide based coupling reaction between mono-*N*-Boc protected phenylendiamines **9**–**11** and benzoic acids **3**–**8** followed by deprotection of the resulting *N*-Boc products to give the final probes in 70–80% overall yield[13] (Scheme 2).

The inhibitory profile of the probes against class I HDAC isoforms was determined using a fluorogenic assay and the results are given in Table 1. The inhibition of HDAC8 was measured using the fluorogenic acetylated substrate Fluor de Lys and purified recombinant human HDAC8 from *Escherichia coli*,[14] whereas the inhibition of HDAC1–3 was measured using fluorogenic acetylated substrate Boc-L-Lys(Ac)-AMC and commercially available recombinant human HDAC1–3.[15] We also explored the effect of preincubation with HDAC1, 2, 3, and 8 as it was previously observed that the potency of the benzamide-based HDAC inhibitors increased with preincubation with HDAC1–3.[11,16] The maximum incubation time was chosen on the basis of stability of HDAC proteins in the conditions used to determine IC_{50} values. We found that for HDAC1, 3, and 8 the maximum incubation time was 3 h, whereas HDAC2 protein was stable for 24 h. The IC_{50} values of ligands **1** and **2** determined in this study vary from those reported previously.[10,11] We attribute this discrepancy to the differences in the assay conditions, the protein sources, substrates, and preincubation times. The analysis of SAR was facilitated by docking all the probes to HDAC2 (PDB:3MAX),[11] HDAC3 (PDB:4A69),[17] and HDAC8 (PDB:1T69)[18] using GOLD v.5.1.[19,20]

All the newly synthesized benzamide-based probes had activity ranging between 70 nM and 55 μM and 110 nM and 77 μM for HDAC1 and HDAC2, respectively. All of the probes demonstrated a robust 2- to 40-fold increase in inhibition of HDAC1 and 2 upon preincubation with the enzymes for 3 h and 24 h, respectively (Table 2). Consistent with the previously reported observation,[11] SAHA, a hydroxamate-based inhibitor, did not exhibit time-dependent inhibition. Similar trends were observed with HDAC3 and HDAC8. In the discussion below we will use only IC_{50}'s obtained at the maximum preincubation time, unless specified otherwise.

In general, the probes exhibited better activity and selectivity for HDAC1 and 2 as compared to HDAC3 and HDAC8 (Table 1). The most HDAC1 and 2 potent probe **1b** had an estimated 100- and 1000-fold selectivity for HDAC1 and 2 as compared to HDAC3 and HDAC8, respectively. In the case of HDAC8, no inhibition was observed after 5 min, whereas inhibition of HDAC3 varied from 2% for **1g** to 21% for **2c** at 10 μM concentration of the inhibitors. After preincubation for 3 h, inhibition of HDAC3 and HDAC8 by the probes varied from 6.5% for **1g** to 56% for **1b** and from 4.7% for **1b** to 26% for **1g**, respectively. Similarly to the probes, ligand **1** showed pronounced inhibition of HDAC3 and HDAC8, 96% and 24%, respectively, and ligand **2** inhibited 40% of activity of HDAC3 and only 3% of activity of HDAC8. Despite the similarity of probes **2a**–**c**, **2a** did not inhibit HDAC8 at 10 μM, whereas both **2b** and **2c** inhibited 25% and 22% of activity of HDAC8, respectively.

Gangloff et al.[11] suggested that the time-dependent inhibition in the case of HDAC2 may be explained by the gradual disruption of the internal hydrogen bond between the aniline hydrogen and carbonyl oxygen in the unbound form of the ligand so as to form a bidentate complex with Zn^{2+} ion in the bound form. After a preincubation 3 h, increase in inhibition of HDAC1 and HDAC2 by the probes varied from 1.6-fold for **1d** to 17-fold for **1b** and from 1.3-fold for **1g** to 7.1-fold for **2c**, respectively, (Table 2). After



Scheme 1. Reagents and conditions: (a) $NaNO_2$, HCl, NaN_3, 5 h, 0 °C–rt, 85%; (b) $SOCl_2$, MeOH, 8 h, 0 °C –rt, 87%; (c) K_2CO_3, 2-azidoethyl-4-methylbenzene sulphonate, acetone, 5 h, reflux, 77%; (d) THF/H_2O (1:1), KOH, 10 h, 70 °C, 92%; (e) NaN_3, Sod. ascorbate, CuI, *N*,*N*-dimethylethane-1,2-diamine, EtOH/H_2O, reflux, 92%; (f) (i) NaN_3, CH_3CN, reflux, 80%; (ii) oxalyl chloride, DCM, 6 h; (g) methyl 4-aminobenzoate, pyridine, DCM, 0 °C–rt, 86%; (h) 2 N NaOH, THF/H_2O (8:2), 2 h, rt, 93%.

Download English Version:

https://daneshyari.com/en/article/1371497

Download Persian Version:

https://daneshyari.com/article/1371497

Daneshyari.com