Bioorganic & Medicinal Chemistry Letters 19 (2009) 2346–2349

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

N-Hydroxy-(4-oxime)-cinnamide: A versatile scaffold for the synthesis of novel histone deacetilase (HDAC) inhibitors

Giuseppe Giannini *, Mauro Marzi, Riccardo Pezzi, Tiziana Brunetti, Gianfranco Battistuzzi, Maria Di Marzo, Walter Cabri, Loredana Vesci, Claudio Pisano

R&D Sigma-Tau S.p.A., Via Pontina, km 30, 400 I-00040 Pomezia, Italy

ARTICLE INFO

Article history:
Received 12 January 2009
Revised 3 February 2009
Accepted 4 February 2009
Available online 12 February 2009

Keywords:
HDAC inhibitors
Cinnamide
Hydroxamic acids
Antitumor

ABSTRACT

With the aim to discover novel HDAC inhibitors with high potency and good safety profiles, we have designed a small library based on a *N*-hydroxy-(4-oxime)-cinnamide scaffold. We describe the synthesis of these novel compounds and some preliminary in vitro cytotoxic activity on three tumor cell lines, NB4, H460 and HCT116, as well as their inhibitory activity against class I, II and IV HDAC. Several 4-oxime derivatives demonstrated a promising inhibitory activity on HDAC6 and HDAC8 coupled to a good selectivity profile.

Histone deacetylases (HDACs) are enzymes involved in the remodeling of chromatin and play a key role in the epigenetic regulation of gene expression. HDACs catalyze the removal of acetyl groups from the lysine residues of core histones and other proteins that control cellular functions such as proliferation, migration, differentiation and cell death. Therefore, inhibition of HDACs represents an actual therapeutic approach to discover new targeted anticancer agents.[1]

The 18 HDACs identified in humans can be subdivided into four classes based on their homology with the ones from yeast, their subcellular localization and their enzymatic activities.

Class I, II and IV HDACs contain a zinc atom in their catalytic site, whereas class III HDACs (sirtuins) have a unique enzymatic mechanism of deacetylation dependent on the cofactor NAD^+ and are virtually unaffected by any of the HDAC inhibitors (HDACi) currently in development.[2,3]

HDACi entered in clinical studies can be divided into several structural classes including hydroxamates, cyclic peptides, aliphatic acids and benzamides.[4] Zolinza® (SAHA, vorinostat), belonging to the hydroxamic acid class, represents the first-in-class drug as pan-HDACi approved by the FDA in October 2006.[5,6]

Actually, there is a great interest in identifying compounds with improved HDAC isoform specificity[7] and many studies are in progress to assess which HDAC isotype(s) should be targeted for anticancer activity.[8]

A number of molecules containing a cinnamoyl hydroxamic acid residue are or have been under clinical investigations as non-selective inhibitors. Those include LAQ-824 (Phase II, retired)[9] **1**, LBH-589 (Phase II, Panobinostat)[10] **2**, PDX-101 (Phase II, Belinostat)[11] **3**, PCI-24781 (Phase I)[12] **4** and SB-939 (Phase I)[13] **5**. Compound **6**[14] is an example of *N*-(2-aminophenyl)-acrylamide with interesting antitumor activity in vivo (Fig. 1).

It is evident that many of these pharmacophores share a *N*-hydroxycinnamide moiety as a common scaffold. The structure fits with the standard modular construction of HDACi (Fig. 2) where

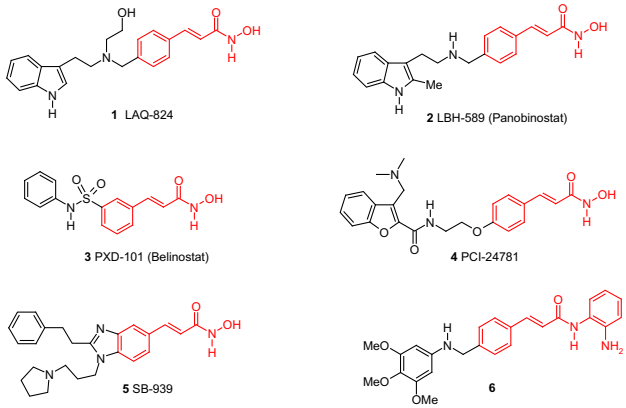


Figure 1. Structure of molecules currently undergoing clinical trials.

* Corresponding author. Tel.: +39 0691393640; fax: +39 0691393638.
E-mail address: giuseppe.giannini@sigma-tau.it (G. Giannini).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.02.029

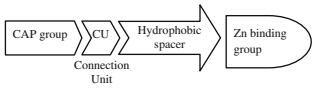


Figure 2. Standard structure of HDACi.

the cinnamide moiety corresponds to the hydrophobic spacer and the hydroxamic acid is used as the Zn binding group (ZBG).[15]

Keeping in mind the structural requirements for this commonly accepted pharmacophore, we designed a small library of compounds (**7**–**21**) bearing few structural modifications of the CAP group, the connecting unit (CU) and the ZBG, starting from the versatile scaffold *N*-hydroxy-(4-oxime)-cinnamide.[16]

4-Oxime-cinnamoyl hydroxamate derivatives **7–14** (Fig. 3) were obtained through modification of the capping group starting from various O-substituted hydroxylamine. Compounds **15**–**16** (Fig. 3) are cinnamic benzamide derivates, synthesized to evaluate alternative ZBG's.

With the aim to explore the influence of the double bond of the cinnamic structure on biological activity, we have also synthesized compounds **17**–**18** (Fig. 4) as analogues of **12**.

We have also explored the influence of the position of the oxime moiety on the aromatic ring (i.e., compound **19** (Fig. 5) vs compound **14**).

Finally, the oxime moiety was incorporated into a ring to form the oxazolidine **20** and oxazoline **21** adducts (Fig. 6).

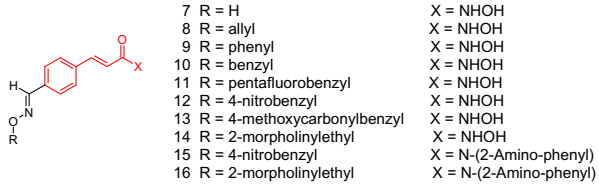


Figure 3. Structure of new 4-oxime-cinnamoyl hydroxamate.

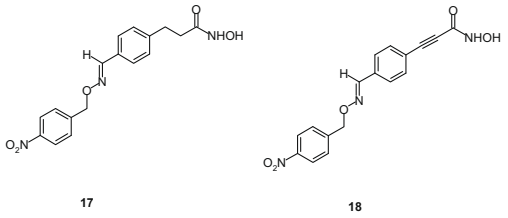


Figure 4. Structure of two analogues of compound **12** obtained through modification of its unsaturation grade.

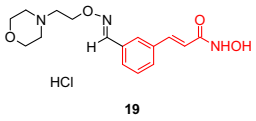


Figure 5. Structure of a *meta*-substituted cinnamoyl hydroxamate derivative.

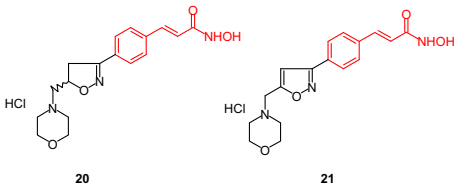


Figure 6. Structure of oxazolidine and oxazoline groups.

The 4-oxime-cinnamoyl hydroxamate derivatives (**7**–**14**) and 4-oxime-cinnamic benzamide (**15**–**16**) (Scheme 1) were synthesized through condensation of *p*-formyl cinnamic acid with various O-substituted hydroxylamines. Intermediates **23** were converted to hydroxamic acids (**7-14**) by coupling with O-protected hydroxylamine via ethyl chloroformate and subsequent deprotection by treatment with HCl in dioxane. Alternatively, intermediates **23** were directly converted to cinnamic benzamide (**15**–**16**) by addition of benzene-1,2-diamine in the presence of PyBOP coupling reagent.

The synthesis of **17**–**18** were similar to the synthetic approach used to achieve 4-oxime-cinnamoyl hydroxamate derivatives synthesis. Compound **17** was obtained via a Heck reaction starting from 4-bromo-benzaldehyde (**24**) and 2,3-diethoxypropene.[17] After basic hydrolysis and condensation with 4-nitro-benzylhydroxylamine hydrochloride, intermediate **26** was obtained. Coupling of **26** with O-protected hydroxylamine using HATU and subsequent TFA-mediated deprotection yielded the desired compound **17** (Scheme 2).

Compound **18** was obtained starting from 4-iodo-benzaldehyde (**28**) under Sonogashira reaction conditions.[18]

Intermediate **29** was converted to compound **30** through condensation with 4-nitro-benzylhydroxylamine hydrocholoride. Coupling with O-protected hydroxylamine and subsequent hydrolysis using Amberlyst 15(R) yielded the desired compound **18** (Scheme 3).

The 3-oxime analogue of compound **14** (compound **19**) was synthesized, starting from *m*-formyl cinnamic acid according to the synthetic approach cited above (Scheme 1).

Oxazolidine **20** and oxazoline **21** groups were synthesized as described precedently[19,20] using allyl alcohol and propargyl alcohol, respectively (Scheme 4).

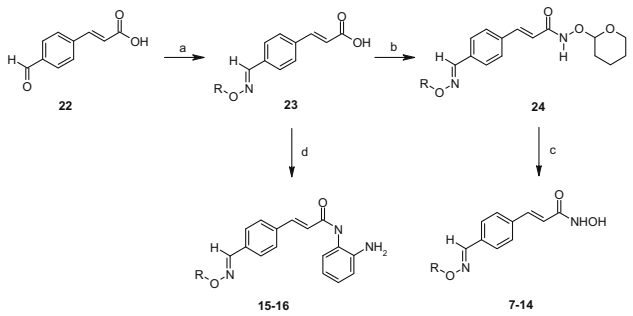
For all these compounds, we evaluated the anti-proliferative activity in vitro using the human cell lines NB4 (promyelocytic leukemia), NCI-H460 (non-small cell lung carcinoma) and HCT116 (colon carcinoma) (Table 1).

Compounds **11–14** and **21** were selected for further studies. Compounds **11–13** were chosen for their significant anti-proliferative activity, compound **14** for its good solubility owing to the morpholine structure and compound **21** to evaluate the effect of replacement of the oxime moiety with an oxazoline group.

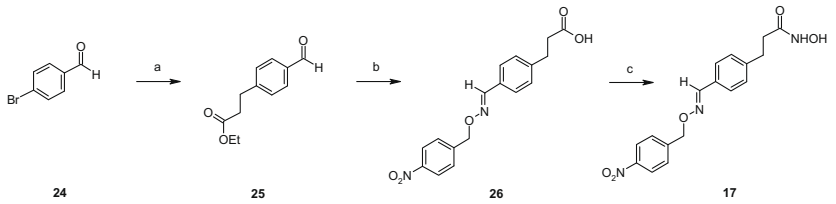
For these selected compounds, we evaluated the in vitro inhibitory activity against 11 HDACs isoforms.

In Table 2 we show the inhibition profile using human HDAC enzymes and a fluorogenic peptide, from p53 residues 379–382 (RHKKAc) at 50 μM, as the substrate.[21]

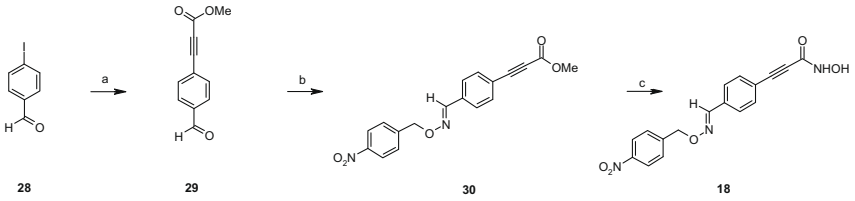
In summary, a versatile scaffold for the synthesis of novel HDAC inhibitors has been investigated.



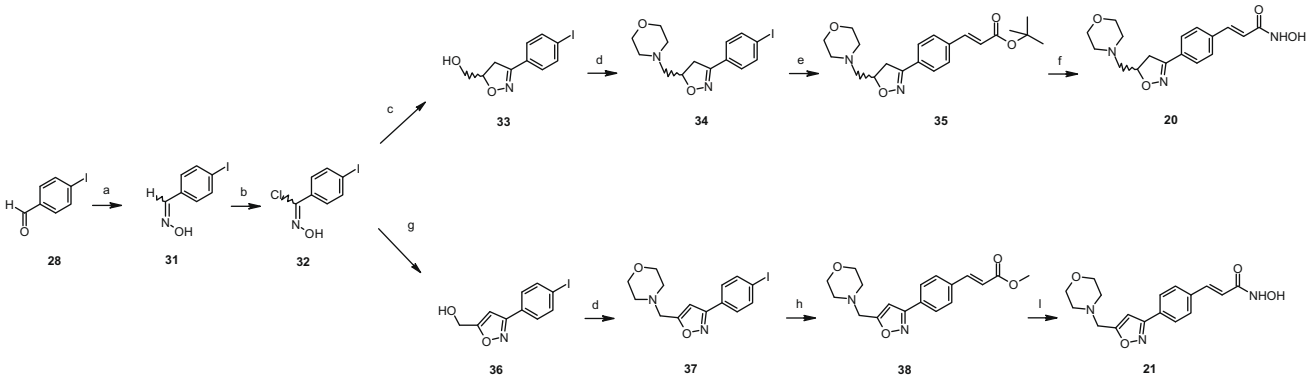
Scheme 1. Reagents and conditions: (a) RO–NH_2·HCl (1.2 equiv), DMF, 18 h, 40–50 °C; (b) (i) ethyl chloroformate (1.2 equiv), TEA (2 equiv), THF_a; (ii) *O*-tetrahydro-2*H*-pyran-2-yl-hydroxylamine (1.2 equiv), 4 h, rt; (c) 4 M HCl in dioxane, 3-18 h, rt; (d) benzene-1,2-diamine (5 equiv), PyBOP (1.1 equiv), DIPEA (3 equiv), DMF, rt.



Scheme 2. Reagents and conditions: (a) 3,3-diethoxy-propene (3 equiv), TBACl (1 equiv), *n*-Bu_4 N (2 equiv), $Pd(OAc)_2$ (0.03 equiv), DMF, 90 °C, overnight (84%); (b) (i) LiOH sol. THF/H_2O 1:1, 50 °C, 3 h (100%); (ii) 4-nitrobenzylhydroxylamine HCl (1.1 equiv), DMF, 50 °C; (c) (i) HATU (1.1 equiv), TEA (2 equiv), $(Ph)_3CONH_2$ (1.1 equiv), DMF, rt (78%); (ii) TFA, DCM (95%).



Scheme 3. Reagents and conditions: (a) methylpropiolate (4 equiv), K_2CO_3 (2 equiv), CuI (0.04 equiv), $PdCl_2(PPh_3)_2$ (0.02 equiv), THF, 70 °C, 12 h (80%); (b) 4-nitrobenzylhydroxylamine HCl, DMF, 50 °C, 18 h (96%); (c) (i) *O*-THP-hydroxylamine (1 equiv), sodium HMDS 1 M THF solution (1 equiv); THF, −78 °C, 2 h (35%); (ii) Amberlyst 15(R), MeOH; 50 °C, 2 h (60%).



Scheme 4. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$ (1 equiv), NaOH (2.5 equiv) sol. 50% w/w H_2O/EtOH = 2/1, rt, 1 h; (b) NCS (1 equiv), DMF, 50 °C, 1 h; (c) allyl alcohol (1.5 equiv), TEA (1.5 equiv), DCM, rt; (d) (i) MsCl (1.1), TEA (1.3), DCM, 1 h, 0 °C to rt; (ii) morpholine (3 equiv), THF, 60 °C, 4 h; (e) *tert*-butyl acrylate (4 equiv), TEA (3 equiv), $PdCl_2(PPh_3)_2$ (0.10 equiv), DMF, N_2, 50 °C, overnight; (f) (i) 4 M HCl in dioxane, THF, 50 °C, 2d; (ii) ethyl chloroformate (1.2 equiv), TEA (10 equiv), NH_2O–THP (1.2 equiv), THF, 0.3 h, 0 °C to rt; (iii) HCl sol. 4 M dioxane, overnight, rt; (g) propargyl alcohol (1.5 equiv), TEA (1.5 equiv), DCM, rt; (h) methyl acrylate (5 equiv), TBAOAc (2 equiv), K_2CO_3 (1.5 equiv), $Pd(OAc)_2$ (0.03 equiv), DMF, Ar, 70 °C, 3 h (91%); (l) (i) MeOH, NaOH 1 M, 3 h, rt (100%); (ii) ethyl chloroformate (1.2 equiv), TEA (10 equiv), NH_2O–THP (1.2 equiv), THF, 0.3 h, 0 °C to rt; (iii) 4 M HCl in dioxane, overnight, rt.

Table 1
In vitro cytotoxic activity of novel *N*-hydroxy-(4-oxime)-cinnamide derivatives for compounds **7**–**21**

Compounds	Cell growth inhibition[a] (IC_{50}, μM[b])		
	NB4	NCI-H460	HCT116
7	2.9	6.3	3.5
8	1.4	3.2	3.5
9	2.7	5.1	3.7
10	0.62	1.6	2.8
11	0.7	1.6	2.4
12	0.76	2.6	1.2
13	0.82	1.7	1.7
14	1.7	2.9	1.8
15	>20	>20	>20
16	8.9	>20	>20
17	0.95	7.7	3.1
18	>20	>20	>20
19	1.9	6.6	4.0
20	nd	9.9	3.0
21	nd	7.0	4.0

[a] Growth inhibition was measured by SRB (sulphorodamine B) assay after 24 h of treatment and 48 h of recovery.
[b] Values are means of three experiments (nd = not determined).

A structure–activity-relationship (SAR) analysis was performed allowing to gather more information about this scaffold in the design of selective HDAC6 and HDAC8 inhibitors.

While selective HDAC8 inhibitors (e.g., CRA-A, PCI-34051, SB-379278A) as well as selective HDAC6 inhibitors (e.g., tubacin, some coumarin and thiolate derivatives) were already known,[7] this study has given a surprising result: the highlighted compounds represent, to the best of our knowledge, a new class of inhibitors active on HDAC6 as well as on HDAC8.

Many other details emerged from our SAR analysis. The requirement of a significant steric hindrance for the CAP groups was not confirmed (**7** vs **8** and **9**) Hydrophilic groups, such as the water-soluble morpholine, confer an interesting biological profile (**14**). The oxime can be enclosed in a cycle (oxazolidine or oxazoline) with scarce influence on cytotoxic and HDACi activities (**14** vs **20** and **21**). In the cinnamic system, substitution in the *para* position is preferred to the *meta* position (**14** vs **19**). Regarding the ZBG, data confirm that the moiety including the hydroxamic acid and the double bond is more potent than the benzamide moiety (**12** and **14** vs **15** and **16**, respectively). Furthermore, the double bond shows to confer more potency than the single or triple bond (**12** vs **17** and **18**, respectively).

Table 2
Compounds **11**, **12**, **13**, **14**, **21** HDAC isoform selectivity profile

Compounds[a]	IC_{50} (μM)										
	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HADAC9	HADC10	HDAC11
11	5.480	11.00	2.800	38.80	7.20	0.661	20.00	1.290	12.00	7.360	5.48
12	0.790	2.67	0.160	2.040	1.16	0.124	2.30	0.070	2.470	1.580	0.790
13	1.820	15.80	0.659	12.90	3.67	0.094	36.30	0.603	6.430	5.430	10.80
14	3.060	27.70	4.080	16.10	7.36	0.303	33.70	0.235	13.10	7.840	3.060
21	6.260	13.2	3.140	11.90	10.80	0.175	22.30	0.127	11.20	6.100	6.260
TSA	0.007	0.023	0.010	0.012	0.016	0.0004	0.022	0.090	0.038	0.020	0.015

[a] Assay condition was started with a 50 μM solution, 10 doses with 1:3 dilution. Trichostatin A (TSA) was used as reference compound.

Among the selected derivatives, compound **12** (ST2987) turned out to be particularly active versus three isoforms: HDAC8, HDAC3 and HDAC6.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.029.

References and notes

1. Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discov.* **2006**, *5*, 769.
2. Dokmanovic, M.; Clarke, C.; Marks, P. A. *Mol. Cancer Res.* **2007**, *510*, 981.
3. Acharya, M. R.; Sparreboom, A.; Venitz, J.; Figg, W. D. *Mol. Pharmacol.* **2005**, *68*, 917.
4. Garber, K. *Nat. Biotechnol.* **2007**, *25*, 17.
5. Grant, S.; Easley, C.; Kirkpatrick, P. *Nat. Rev. Drug Discov.* **2007**, *6*, 21.
6. Marks, P. A.; Breslow, R. *Nat. Biotechnol.* **2007**, *25*, 84.
7. Bieliauskas, A. V.; Pflum, M. K. H. *Chem. Soc. Rev.* **2008**, *37*, 1402.
8. Karagiannis, T. C.; El-Osta, A. *Leukemia* **2007**, *21*, 61.
9. Remiszewski, S. W. *Curr. Med. Chem.* **2003**, *10*, 2393.
10. Rasheed, W. K.; Johnstone, R. W.; Prince, H. M. *Expert Opin. Invest. Drugs* **2007**, *16*, 659.
11. Plumb, J. A.; Finn, P. W.; Williams, R. J.; Bandara, M. J.; Romero, M. R.; Watkins, C. J.; La Thangue, N. B.; Brown, R. *Mol. Cancer Ther.* **2003**, *2*, 721.
12. Uggy, J. J.; Cao, Z. A.; Bass, K. E.; Verner, E.; Balasubramanian, S.; Liu, L.; Schultz, B. E.; Young, P. R.; Dalrymple, S. A. *Mol. Cancer Ther.* **2006**, *55*, 1309.
13. Chen, D.; Deng, W.; Lee, K. C. L.; Lye, P. L.; Sun, E. T.; Wang, H.; Yu, N. WO2007030080 A1.
14. Moradei, O.; Leit, S.; Zhou, N.; Fréchette, S.; Paquin, I.; Raeppel, S.; Gaudette, F.; Bouchain, G.; Woo, S. H.; Vaisburg, A.; Fournel, M.; Kalita, A.; Lu, A.; Trachy-Bourget, M.; Yan, P.; Liu, J.; Li, Z.; Rahil, J.; MacLeod, A. R.; Besterman, J. M.; Delorme, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4048.
15. Mai, A.; Massa, S.; Rotili, D.; Simeoni, S.; Ragno, R.; Botta, G.; Nebbioso, A.; Miceli, M.; Altucci, L.; Brosch, G. *J. Med. Chem.* **2006**, *49*, 6046.
16. Pisano, C.; Giannini, G.; Battistuzzi, G.; Di Marzo, M.; Pezzi, R.; Marzi, M.; Vesci, L.; Zunino, F. WO2006131482.
17. Battistuzzi, G.; Cacchi, S.; Fabrizi, G.; Bernini, R. *Synlett* **2003**, 1133.
18. InsEckert, T.; Ipaktschi, R. *Synth. Commun.* **1998**, *28*, 327.
19. Barbachyn, M. R.; Cleek, G. J.; Dolak, L. A.; Garmon, S. A.; Morris, J.; Seest, E. P.; Thomas, R. C.; Toops, D. S.; Watt, W.; Wishka, D. G.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H.; Adams, W. J.; Friis, J. M.; Slatter, J. G.; Sams, J. P.; Oien, N. L.; Zaya, M. J.; Wienkers, L. C.; Wynalda, M. A. *J. Med. Chem.* **2003**, *46*, 284.
20. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.
21. Screening performed by Reaction Biology Corp using trichostatin A (TSA) as reference compound.