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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4784–4787

# Exploring alternative Zn-binding groups in the design of HDAC inhibitors: Squaric acid, *N*-hydroxyurea, and oxazoline analogues of SAHA

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Received 21 June 2006; accepted 26 June 2006
Available online 25 July 2006

**Abstract**—Analogues of suberoylanilide hydroxamic acid (SAHA) were prepared by replacing the Zn-binding group with squaric acid, *N*-hydroxyurea, and 4-hydroxymethyl oxazoline units, also varying the length of the aliphatic chain. No inhibitory activity on HDAC was observed below 1.0 μM and no cytotoxic activity on different tumor cell lines was seen below 20.0 μM.


HDACs are nuclear enzymes that play a major role in regulating gene expression.[1] They catalyze the deacetylation of the *N*-acetyl lysine residues thereby changing the accessibility of transcription factors to DNA, thus affecting the chromatin remodeling process. Cell-specific patterns of gene expression depending on histone acetylation result from a balance of the competing activities of two classes of enzymes, the histone acetyl transferases (HATs) and the histone deacetylases (HDACs). Perturbation of this balance has been linked to cancer, and inhibition of HDAC has been shown to have antiproliferative effects on tumor cell lines, resulting in considerable interest in this field.[2]

The list of known inhibitors of HDACs covers a wide cross-section of structures including natural products such as Trichostatin A (TSA)[3] and unnatural surrogates such as suberoylanilide hydroxamic acid (SAHA)[4] (Fig. 1). A large body of work has been devoted to the synthesis of hydroxamic acid analogues of acyclic and heterocyclic compounds.

In spite of the promising results, the potential toxicity of hydroxamic acids has also instigated the search for other zinc-binding groups (ZBGs) that can be incorporated in the structures of metalloproteases inhibitors in general.[5]

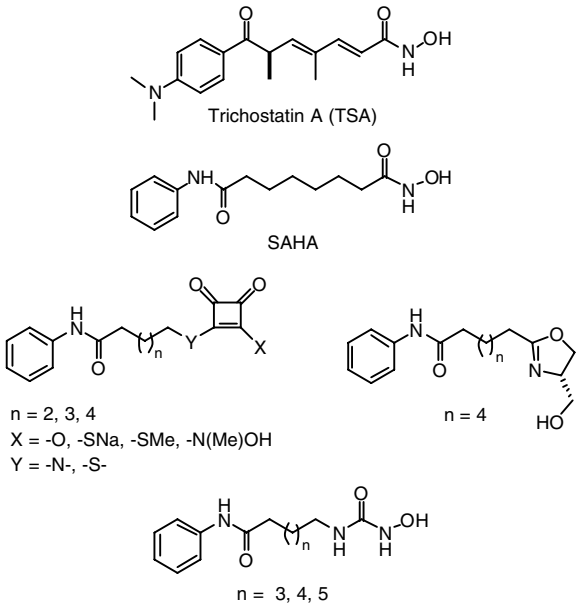


**Figure 1.** Structures of known inhibitors and proposed prototypes.

*Keywords*: Metalloprotease inhibitor.
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doi:10.1016/j.bmcl.2006.06.090

We wish to report on our efforts to prepare analogues of SAHA in which the hydroxamic acid residue has been replaced by squaric acid, *N*-hydroxyurea, and hydroxymethyl oxazoline units individually (Fig. 1). To the best of our knowledge, these well-known motifs have not been synthesized and tested as non-hydroxamate HDAC inhibitors.[6]

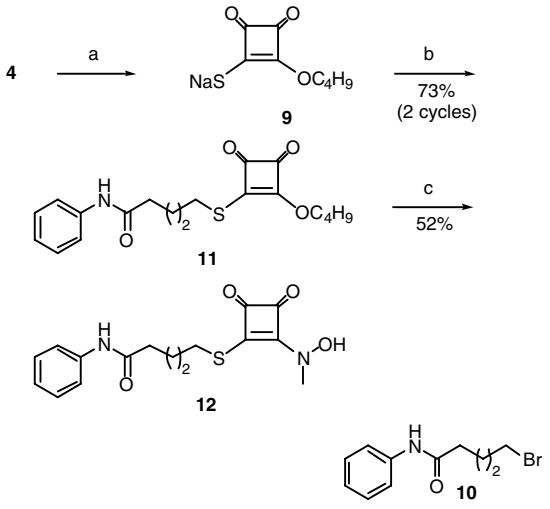
Being cognizant that the zinc-binding geometry of hydroxamic acids[7] may be different compared to squaric acids or the *N*-hydroxyurea units, we also varied the length of the aliphatic chain to allow for greater flexibility and accommodation within the active site domain.[8]

*Squaric acid derivatives.* Since the first synthesis of squaric acid in 1959,[9] a number of derivatives have been reported and their chemistry has been well studied in different contexts over the years.[10,11] Squaric acid-based inhibitors of matrix metalloproteases were recently reported.[12] Due to its different resonance forms, squaric acid can mimic acidic functions, which was the main design element in this study, although it was not clear whether the spatial requirements could be satisfied in the active site of HDAC enzymes.[8]

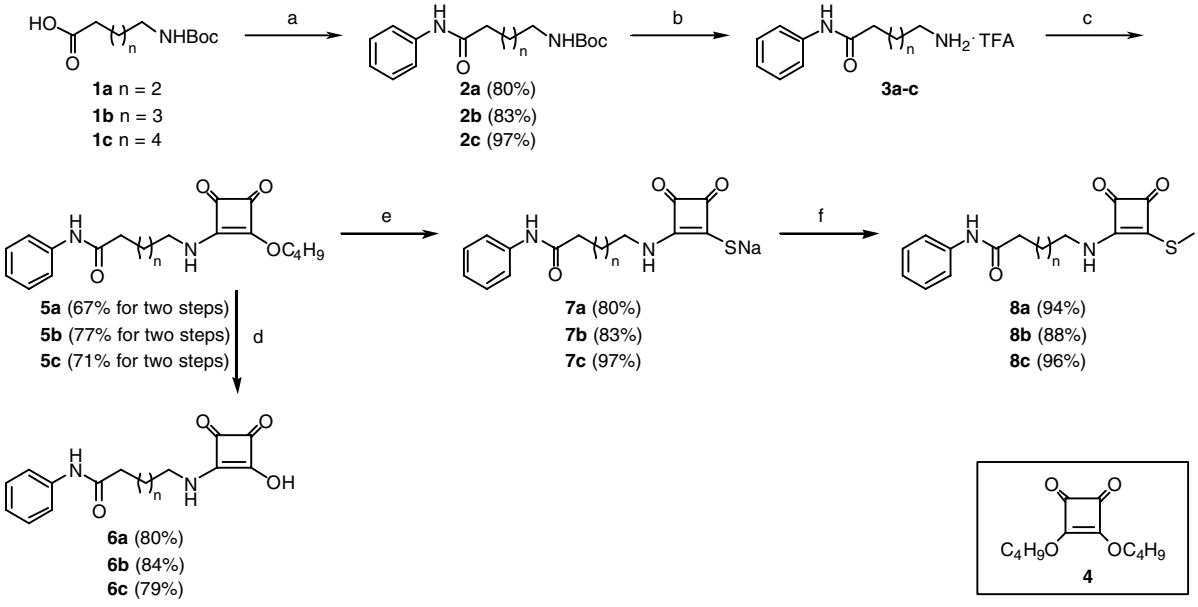
We therefore focused first on the synthesis of SAHA-type analogues of different chain lengths and harboring a squaric acid unit that contains exocyclic nitrogen or sulfur groups to simulate vinylogous hydroxamic acids and thioether counterparts as potential zinc binders. A series of *N*-Boc $\omega$-amino carboxylic acids **1a**–**c** was converted to the corresponding phenylamides **2a**–**c**, and then cleaved to the amines **3a**–**c** (Scheme 1). Treatment with the readily available di-*n*-butyl squarate **4** led to the corresponding monoamides **5a**–**c** in good yields.[13] Mineral acid hydrolysis gave the corresponding squaric acid SAHA analogues **6a**–**c** in excellent yields.[14]

Alternatively, treatment of the esters **5a**–**c** with aqueous sodium hydrogen sulfide in ethanol afforded the corresponding thiol monoacids as their sodium salts **7a**–**c**.[13] The methylthioesters **8a**–**c** were obtained upon treatment with methyl iodide (Scheme 1).

Alternatively, intermediate **4** was converted into the corresponding monosodium thiolate by treatment with sodium hydrogen sulfide in methanol and then coupled with the readily available bromide **10** (prepared in two steps from $\delta$-valerolactone)[6,15] in DMF, to afford **11** (Scheme 2). Treatment with *N*-methyl hydroxylamine gave the thio squaric *N*-methyl hydroxamate analogue **12**.




**Scheme 2.** Reagents: (a) $NaHS{\cdot}H_2O$, MeOH; (b) **10**, DMF; (c) MeNHOH·HCl, NaOH, MeOH.



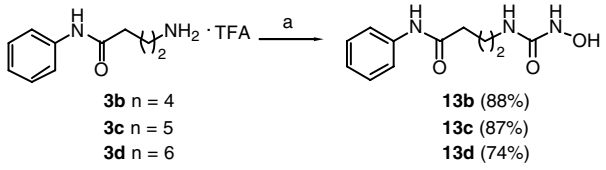

**Scheme 1.** Reagents and conditions: (a) $PhNH_2$, PyBop, $Et_3N$, $CH_2Cl_2$; (b) TFA, $CH_2Cl_2$, 0 °C to rt; (c) **4**, $Et_3N$, EtOH; (d) 4 N HCl/dioxane, $H_2O$; (e) aq NaHS, EtOH; (f) MeI.

*N-Hydroxyurea derivatives*. The replacement of a hydroxamic acid moiety with an *N*-hydroxyurea counterpart has received only scant attention in the search for HDAC inhibitors. In principle, hydroxamic acids should be more acidic compared to an *N*-hydroxyurea, although the requisite terminal functionality to bind the zinc atom is present in both.

Suzuki and coworkers[6] recently reported that a SAHA *N*-hydroxyurea derivative was weakly active as an inhibitor of HDAC. Our intention was to probe the influence of the hydrocarbon chain length, knowing that the active site domain of SAHA and TSA can accommodate a limited number of carbon atoms in an acyclic chain.[5a,8]

Treatment of the readily available amines **3b–d** with hydroxylamine in the presence of carbonyl diimidazole led to the intended analogues **13b–d** in very good overall yields (Scheme 3).

Ether and amide analogues of SAHA have been recently disclosed in a patent.[16] We therefore prepared an ether prototype in the *N*-hydroxyurea series. Briefly, (±)-aminopimelic acid **14** was selectively protected as the lactone acetal **15**, diazotized, and the resulting alcohol then chain-extended via an Arndt-Eistert reaction (Scheme 4).[17]

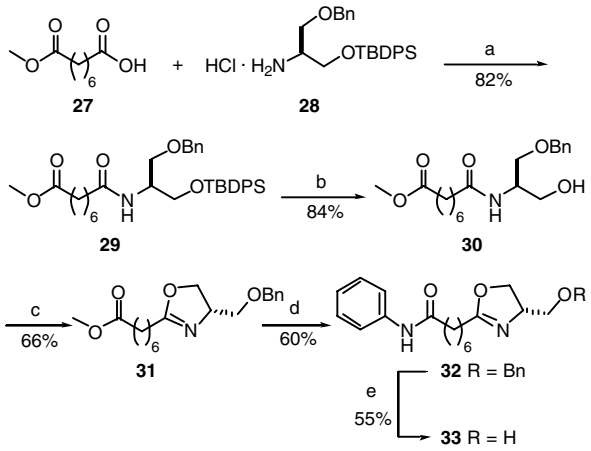



**Scheme 3.** Reagents: (a) CDI, $Et_3N$, $NH_2OH{\cdot}HCl$, $CH_2Cl_2$.

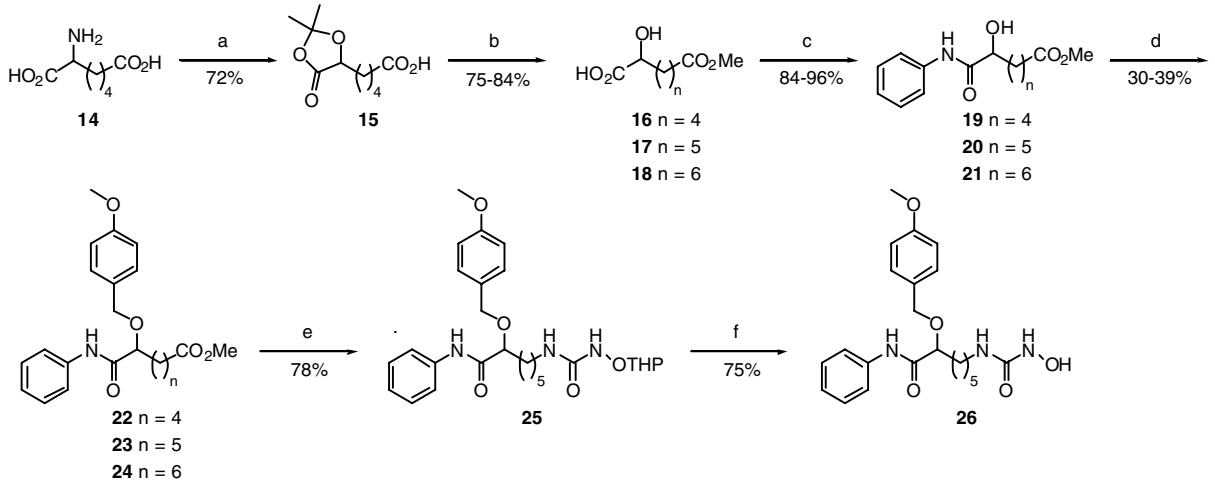
A series of resulting hydroxy acids **16–18** were converted to the corresponding anilides **19–21**, which were O-alkylated to give *O*-PMB ethers **22–24**. Intermediate **23** ($n = 5$) was converted into the corresponding *N*-OTHP *N*-hydroxyurea **25** and the latter subjected to mild acid hydrolysis to afford **26**.

*Hydroxymethyl oxazolines*. Cook and coworkers[18] have explored the core of 4-hydroxymethyl oxazolines as potential MMP inhibitors. We therefore prepared SAHA analogues in which the hydroxamic acid group was replaced with this moiety as shown in Scheme 5.

Amide **29** was prepared in very good yields starting from monomethyl suberate **27** and the chiron **28** (readily available from L-serine). Subsequent treatment of **29**




**Scheme 5.** Reagents and conditions: (a) HATU, DIEA, DMAP, $CH_2Cl_2$. (b) TBAF, THF. (c) DAST, $K_2CO_3$, $CH_2Cl_2$, −78 °C. (d) (i) $LiOH{\cdot}H_2O$, MeOH, $H_2O$, THF; (ii) $PhNH_2$, PyBOP, $Et_3N$, DMAP, DMF. (e) $BCl_3$, $CH_2Cl_2$, −40 °C.




**Scheme 4.** Reagents and conditions: (a) (i) $NaNO_2$, 2 N HCl, 0 °C to rt; (ii) 2,2-dimethoxypropane, PTSA. (b) For $n = 4$: (i) $CH_2N_2{\cdot}Et_2O$; (ii) 70% AcOH, 60 °C, 84% for two steps; for $n = 5$: (i) $SOCl_2$, cat. DMF, $CH_2Cl_2$, −10 °C to rt, then −5 °C, $CH_2N_2$, $Et_2O$, rt, 86% for two steps; (ii) AgOBz, $Et_3N$, MeOH, −25 °C to rt, 99%; for $n = 6$: (i) $BH_3{\cdot}DMS$, THF, 0 °C to rt; (ii) $(COCl)_2$, DMSO, $CH_2Cl_2$, −78 °C, then $Et_3N$, rt; (iii) $Ph_3P{=}CHCO_2Me$, $CH_2Cl_2$, 82% for three steps; (iv) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH, 0 °C to rt; (v) 70% AcOH, 65 °C, 92% for two steps. (c) $C_6H_5N{=}S{=}O$, 1,2,4-triazole, $CH_2Cl_2$, 0 °C to rt; (d) $PMBO(C{=}NH)CCl_3$, cat. $BF_3{\cdot}Et_2O$, $CH_2Cl_2$–$Et_2O$. (e) (i) NaOH–THF–MeOH; (ii) diphenyl phosphoryl azide, $Et_3N$, toluene, reflux, then $THPONH_2$, reflux; (f) HCl–MeOH, THF.

with TBAF afforded **30** which underwent DAST-mediated ring-closure to give **31** in very good yields.

Saponification of the methyl ester and introduction of the anilide moiety led to **32** which finally underwent debenzylation by $BCl_3$ in $CH_2Cl_2$ to afford the oxazolidine analogue **33**.

Unfortunately, none of the new analogues in the series exhibited HDAC inhibitory activity below 1.0 μM. Furthermore, no cytotoxic activity on different tumor cell lines was seen below 20.0 μM.

## Acknowledgments

We thank Consiglio Nazionale delle Ricerche, Istituto di Chimica Biomolecolare, Italy, for a sabbatical leave to L.A. and Sigma-Tau Industrie Farmaceutiche Riunite S.p.A. for biological testing.

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