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Identification of a potent and stable antiproliferative agent by the prodrug formation of a thiolate histone deacetylase inhibitor

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Abstract—To identify prodrugs of a thiolate histone deacetylase inhibitor NCH-31 that show potent antiproliferative activity and are stable in human plasma, we synthesized several candidate prodrugs of NCH-31. Among these compounds, *S*-2-methyl-3-phenylpropanoyl compound **2** showed more potent antiproliferative activity and higher stability in human plasma than *S*-isobutyryl compound NCH-51.

The dynamic homeostasis of the nuclear acetylation of histones is regulated by the opposing activity of the enzymes histone acetyl transferases and histone deacetylases (HDACs).[1,2] Deacetylation of histone lysine residues is associated with a condensed chromatin structure resulting in transcriptional repression, whereas acetylation of histones is associated with a more open chromatin configuration and activation of transcription.[3] Aberrant activation of HDACs results in the transcriptional repression of oncoprotein and is linked to the malignant phenotypes of tumors.[4,5] Inhibition of HDACs causes histone hyperacetylation which leads to the disruption of the chromatin structure and the transcriptional activation of genes associated with cancer.[3,6] In addition, it has recently been reported that inhibition of an HDAC exerts antitumor effects through the non-transcriptional pathway in addition to the intervention of transcriptional regulation.[7,8] Indeed, HDAC inhibitors such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (Fig. 1) have potent anticancer effects in vitro and in vivo.[9,10]

In the course of our study of HDAC inhibitors, we found that a series of thiol-based analogues, including NCH-31 (Fig. 2), are potent HDAC inhibitors.[11] Thiols are thought to inhibit HDACs by coordinating the zinc ion in the active site which is required for deacetylation of the acetylated lysine substrate. Further, the *S*-isobutyryl prodrug NCH-51 (Fig. 2), which is thought to be hydrolyzed to free thiol within cells, showed antiproliferative activity against cancer cells,[12] however, NCH-51 has stability problems. NCH-51 was vulnerable to human plasma metabolism at the remaining rate of approximately 50% after 24 h of incubation. We therefore decided to search for prodrugs that are more stable in human plasma and exert more potent antiproliferative activity than NCH-51.

Since prodrugs with a less hindered acyl group tended to exhibit less potent antiproliferative activity,[12] we designed *S*-acyl prodrugs **1**–**4** which bear an acyl group more bulky than NCH-51. Four candidate prodrugs of NCH-31 were synthesized as shown in Scheme 1. The sulfhydryl group of NCH-31 was acylated with the corresponding carboxylic acid to give the desired compounds **1**–**4**.

The compounds synthesized in this study were initially tested in antiproliferative activity assays using human lung cancer NCI-H460 cells and human breast cancer MDA-MB-231 cells (Table 1).[13] Although *S*-2-methoxy-2-phenylacetyl compound **3** and 3-phenyl-2-(phenylmethyl)propanoyl compound **4** were less active than thiol NCH-31 and its *S*-isobutyryl prodrug NCH-51,

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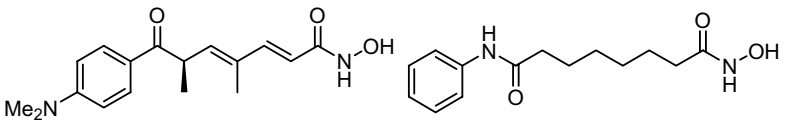
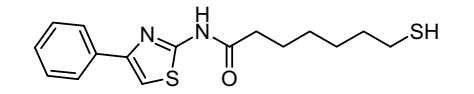
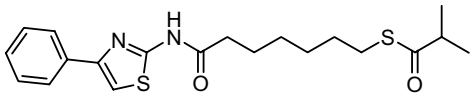


TSA **SAHA**

Figure 1. Structures of TSA and SAHA.

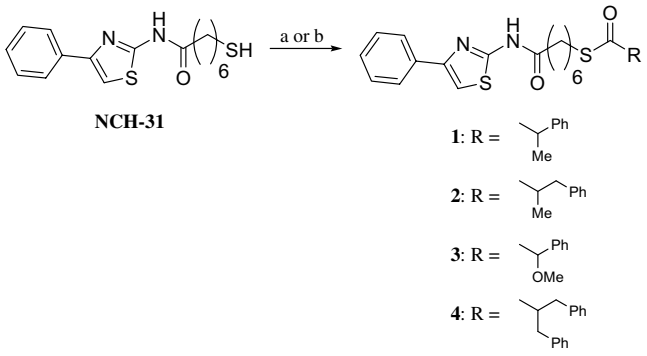


NCH-31



NCH-51

Figure 2. Structures of NCH-31 and NCH-51.

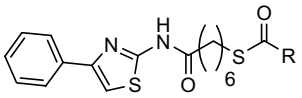


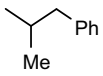
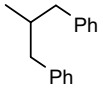
Scheme 1. Reagents and conditions: (a) RCOCl (for **1** and **4**), DMAP, pyridine, 0 °C to rt, 41% for **1**, 54% for **4**; (b) RCOOH (for **2** and **3**), EDCI, DMAP, DMF, rt, 63% for **2**, 23% for **3**.

S-2-phenylpropanoyl compound **1** showed similar activity against NCI-H460 cells and *S*-2-methyl-3-phenylpropanoyl compound **2** also displayed similar activity against both NCI-H460 cells and MDA-MB-231 cells when compared with NCH-51. Furthermore, we examined proliferation inhibition by NCH-51, compounds **1** and **2**, against eight solid cancer cell lines. As can be seen from Table 2, compound **2** showed potent proliferation inhibition against various cancer cells representing a 2-fold improvement over NCH-51 (average EC_{50} of **2** 2.4 μM, NCH-51 4.9 μM).

To investigate the difference in activity between (*R*)-**2** and (*S*)-**2**, optically active (*R*)-**2** and (*S*)-**2** were prepared from (*S*)-4,5,5-trimethyloxazolidin-2-one **5**[14] as outlined in Scheme 2. The chiral auxiliary **5** was converted to compounds **6** and **7** by *N*-propionylation and *N*-3-phenylpropionylation, respectively. Stereoselective enolate benzylation of **6** and methylation of **7**, and the subsequent hydrolysis of **8** and **9** gave optically active 2-methyl-3-phenylpropanoic acids (*R*)-**10** and (*S*)-**10**, respectively. The condensation of carboxylic acids

Table 1. Proliferation inhibition data on NCI-H460 cells and MDA-MB-231 cells for NCH-31, NCH-51, and compounds **1**–**4**[a]



Compound	R	EC_{50} (μM)	
		NCI-H460	MDA-MB-231
NCH-31	–H	7.6	>20
NCH-51		2.1	4.4
1		3.2	>20
2		1.5	1.9
3		11	>20
4		>20	>20

[a] Values are means of at least two experiments.

(*R*)-**10** and (*S*)-**10** with NCH-31 in the presence of EDCI and DMAP afforded optically active (*R*)-**2** and (*S*)-**2**, respectively. The enantiomeric excess of both (*R*)-**2** and (*S*)-**2** was determined to be 90% by chiral column chromatography.[15]

We examined the antiproliferative activity of (*R*)-**2** and (*S*)-**2** against four cancer cell lines, and there was not much difference between the activities of the two stereoisomers (Table 3).

Next, we investigated the in vitro HDAC inhibitory activity of compound **2** (Table 4).[16] Although NCH-31 exhibited potent inhibitory activity against HDAC1 (IC_{50} = 0.048 μM), the *S*-2-methyl-3-phenylpropanoyl prodrug **2** did not inhibit HDAC1 at a concentration of 100 μM.

Compound **2** was evaluated for the accumulation of acetylated histone H4 using Western blot analysis (Fig. 3).[17] Treatment of HCT116 cells with compound **2** produced an increase in the accumulation of acetylated histone H4, which indicated that the antiproliferative activity of compound **2** significantly correlates with the inhibition of HDACs. Since *S*-2-methyl-3-phenylpropanoyl prodrug **2** was totally inactive in an enzyme assay