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Novel Histone Deacetylase Inhibitors: *N*-Hydroxycarboxamides Possessing a Terminal Bicyclic Aryl Group

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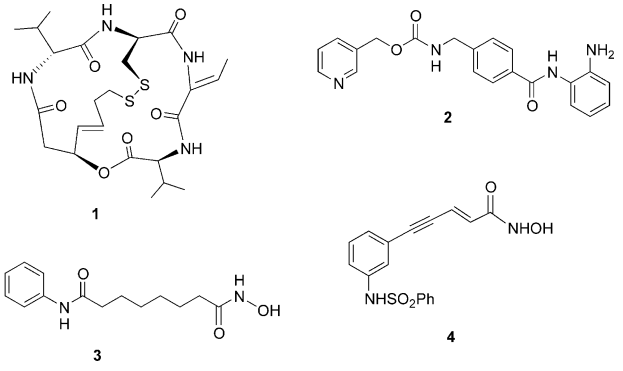
Abstract—Utilizing tranexamic acid as a starting material, a series of *N*-hydroxycarboxamides were synthesized in order to seek new histone deacetylase (HDAC) inhibitors. Further structure optimization involving the replacement of the 1,4-cyclohexylene group with the 1,4-phenylene group yielded the promising HDAC inhibitors which possess a terminal bicyclic aryl amide.

Histone deacetylase (HDAC) inhibitors modulate the deacetylation of ε-*N*-acetyl groups of L-lysine residues in the N-terminal tails of core histones.[1] As HDAC inhibitors from natural resources, trichostatin A[2] (TSA), trapoxin[3] and FK228 (**1**)[4] were isolated; they inhibited strongly HDACs and induce differentiation of cancer cell lines and suppress cell proliferation, respectively. The former two compounds, however, have not yet been applied for medical treatments owing to their instability and toxicity. Afterwards, synthetic inhibitors: MS-275 (**2**),[5] SAHA (**3**)[6] and oxamflatin (**4**)[7] were reported as cancer therapeutic candidates. Compounds **1**, **2** and **3** are now under clinical investigation. In our exploratory studies of new HDAC inhibitors, a series of *N*-hydroxycarboxamides were synthesized utilizing tranexamic acid (TA) as a lead substance since it is well known to be the L-lysine analogue inhibiting fibrinolysis by blockage of L-lysine binding site at plasminogen molecule. Structure optimization by replacing the *trans*-1,4-cyclohexylene group of TA with 1,4-phenylene group and further elaboration yielded promising compounds which represent significant inhibitory activities against cancer cell proliferation and HDACs.



N-hydroxycarboxamides (**9a**–**9d**, **13a**–**13b** and **17**) having the *trans*-1,4-cyclohexylene group were synthesized through Route A, B or C as outlined in Scheme 1. Route A: TA methyl ester hydrochloride (**5**) was treated with aryl acid chloride in the presence of triethylamine (TEA) or with aryl acid in the presence of Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) and TEA, yielding aryl amide (**7**). This compound, after alkaline hydrolysis to acid, was condensed with benzyloxyamine hydrochloride in the presence of BOP-Cl and TEA followed by catalytic hydrogenation of the product to afford target *N*-hydroxycarboxamide (**9**). Route B: Compound **5** was reacted with aryl sulfonyl chloride in the presence of TEA to afford sulfonamide (**11**), which was in turn treated in the same way as for Route A to furnish *N*-hydroxycarboxamide having a terminal phenyl sulfonamide (**13**). Route C: Compound **17** was

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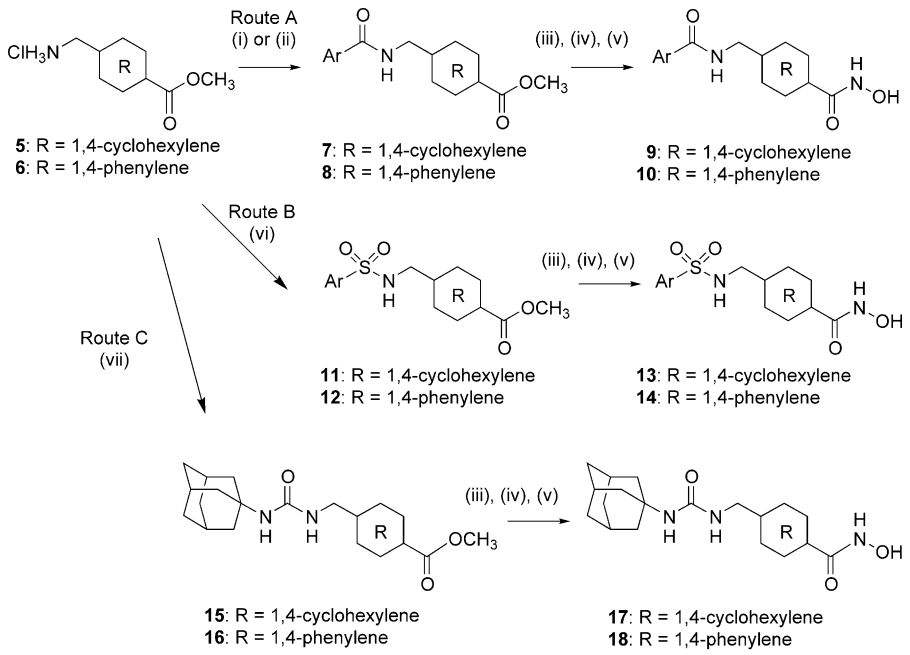


Scheme 1. Conditions: (i) ArCOCl, TEA/THF or CH_2Cl_2; (ii) ArCOOH, BOP-Cl, TEA/CH_2Cl_2 or DMF; (iii) 1 M LiOH/H_2O–THF; (iv) H_2N-OBn, BOP-Cl, TEA/CH_2Cl_2 or DMF; (v) H_2/10%Pd-C/MeOH; (vi) $ArSO_2Cl$, TEA/THF; (vii) 1-adamantylamine, triphosgene, TEA/CH_2Cl_2.

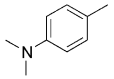
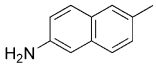
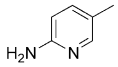
prepared from condensation of **5** and 1-adamantylamine using triphosgene and TEA following the aforementioned conventional steps. Synthetic routes for *N*-hydroxycarboxamides (**10a**–**10f**, **14a**–**14b** and **18**) possessing the 1,4-phenylene group were also shown in Scheme 1. Thus, compound **10** was prepared through Route A starting from condensation of 4-aminomethylbenzoic acid methyl ester hydrochloride (**6**) with aryl acid chloride or with aryl acid to give aryl amide (**8**). However, compounds **10e** and **10f**, having the amino group, were obtained by treating the corresponding Boc-protected compounds (formed through Route A) further with 4M HCl/MeOH, respectively. In the same way, compound **14** was prepared via **12** according to the Route B, whereas compound **18** was formed via **16** according to the Route C.

First of all, the 1,4-cyclohexylene-series compounds (**9a**–**9d**) were estimated for antiproliferative activities against HCT 116 colorectal human carcinoma cells using WST-1 assays:[8] compounds **9a**, **9b** and **9d** represented the activities (7.6, 6.6 and 4.7 μM) of almost comparable magnitude in the IC_{50} values with that of MS-275 (5.4 μM),[9] respectively. Of the two sulfonamides, **13b** comprising the 2-naphthyl was 8-fold more active (IC_{50} 9.4 μM) than **13a** possessing the phenyl though the former was less potent than **9b**. As for the 1,4-phenylene-series compounds (**10a**–**10f**), compounds **10a**, **10c** and **10e**, all having the terminal bicylic aryl (2-naphthyl, 1,4-biphenyl and 6-amino-2-naphthyl groups) had higher inhibitory activities (IC_{50} 0.7, 3.2 and 3.9 μM) relative to the 1,4-cyclohexylene-series compounds, respectively. However, the sulfonamide **14b** possessing the 2-naphthyl had only weak activity (22.5 μM). Interestingly, compounds **9b**, **10a** and **10c** were found to be over 13-fold more potent than the respective regioisomers **9c**, **10b** and **10d**, respectively. These results suggested that topology as well as bulkiness or π electron number in the terminal bicyclic aryl moieties would affect the anfiproliferative activities. Replacement of the 2-naphthyl amide in **10a** with the 2-naphthyl sulfonamide in **14b** reduced the activity. Furthermore, compounds **17** and **18** having the 1-adamantylureido group showed only weak activities.

Second, the compounds showing significant antiprolierative activities (IC_{50}'s ranging between 0.7 and 9.7 μM) against HCT 116 were selected and subjected to the HDAC inhibition tests (Table 1). In the experiments, the IC_{50} values were measured according to the method[2] by Yoshida et al., but using partially purified HDACs from human T cell leukemia Jurkat cell[10] in place of mouse mammary tumor cell line FM3A. Attention has been denoted to the 1,4-phenylene-series compounds **10a** and **10e**, which exhibited the 50% HDAC inhibition at the concentrations of 44 and 39 nM, respectively. These values were almost equal to that of oxamflatin (**4**) (data not shown). It is likely as shown in Figure 1 that **10a** is incorporated into the active-site pocket of the HDAC wherein the hydroxamic acid chelates the zinc ion positioned at the bottom. We speculated that their 1,4-phenylene group could contact to aromatic and hydrophobic amino acid residues at the wall of the pocket, whereas the terminal 2-naphthyl moiety would not only pack the molecule as a cap, but also interact with aryl groups of amino acid residues around the rim.

Compounds **9a**, **9b**, **9d**, **10a**, **10c**, **10e** and **17** were evaluated for antitumor activities using a human cancer cell

Table 1. Inhibition of HCT 116 proliferation and of HDACs[a]

Compd	Ar	WST-1 IC_{50} (μM)[b]	HDACs IC_{50} (nM)[b]
9a		7.6	1500
9b	2-Naph	6.6	2000
9c	1-Naph	90.1	
9d	1,4-Biph	4.7	310
10a	2-Naph	0.7	44
10b	1-Naph	9.7	830
10c	1,4-Biph	3.2	240
10d	1,2-Biph	87.6	
10e		3.9	39
10f		87.2	
13a	Ph	77.9	
13b	2-Naph	9.4	1100
14a	Ph	38.8	
14b	2-Naph	22.5	
17		20.9	
18		112.5	
TSA		—	3.3

[a]Values are means of three experiments.
[b]Concentration required to inhibit by 50%.

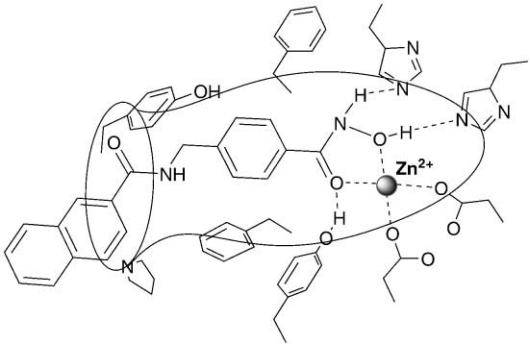


Figure 1. Proposed interaction mode of **10a** and active-site of HDAC.

line panel with database analysis.[11] The respective average concentrations of these compounds required for 50% growth inhibition (GI_{50}) against a panel of 39 cell lines were as follows: 20.4, 6.5, 3.6, 0.81, 4.1, 2.7 and 20.4 μM. Furthermore, the COMPARE analysis[11] represented the correlation coefficients (r) less than 0.75 except for **17**, respectively. Especially, **10a** and **10c** showed the values less than 0.5, thus leading us to expect that they would have a unique action mode.

We have a plan to examine compounds **9a**, **9b**, **9d**, **10a**, **10c** and **10e** for antitumor activities against cancer xenografts in nude mice.

Acknowledgements

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References and Notes

1. (a) Grunstein, M. *Nature (London)* **1997**, *389*, 349. (b) Struhl, K. *Genes Dev*. **1998**, *12*, 599.
2. Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem*. **1990**, *265*, 17174.
3. Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. *J. Biol. Chem*. **1993**, *268*, 22429.
4. Nakajima, H.; Kim, Y. B.; Terano, H.; Yoshida, M.; Horinouchi, S. *Exp. Cell Res*. **1998**, *241*, 126.
5. Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, Y.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 4592.
6. Bulter, L. M.; Agus, D. B.; Scher, H. L.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T.; Rifkind, R. A.; Marks, P.; Richon, V. M. *Cancer Res*. **2000**, *60*, 5165.
7. Kim, Y. B.; Lee, K. H.; Sugita, K.; Yoshida, M.; Horinouchi, S. *Oncogene* **1999**, *18*, 2461.
8. Takenouchi, T.; Munekata, E. *Life Sciences* **1995**, *56*, 479.
9. This compound was synthesized in our laboratory for the HCT 116 test.
10. Mori, H.; Sakamoto, K.; Tsurumi, Y.; Takase, S.; Hino, M. WO 00/21979, 2000.
11. Yamori, T.; Matsunaga, A.; Sato, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuda, Y.; Takezawa, K.; Nakanishi, O.; Kohono, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Turuo, T. *Cancer Res*. **1999**, *59*, 4042 COMPARE analysis is used to evaluate the statistical correlation and estimate the action mode for a test compound through comparison of its differential growth inhibition pattern (finger print) with those of 200 standard anticancer drugs in the database. The correlation coefficient (r) less than 0.5 suggests a new action mode.