SHORT COMMUNICATION

Tetrahydroisoquinolines as novel histone deacetylase inhibitors for treatment of cancer

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Abstract Histone acetylation is a critical process in the regulation of chromatin structure and gene expression. Histone deacetylases (HDACs) remove the acetyl group, leading to chromatin condensation and transcriptional repression. HDAC inhibitors are considered a new class of anticancer agents and have been shown to alter gene transcription and exert antitumor effects. This paper describes our work on the structural determination and structure-activity relationship (SAR) optimization of tetrahydroisoquinoline compounds as HDAC inhibitors. These compounds were tested for their ability to inhibit HDAC 1, 3, 6 and for their ability to inhibit the proliferation of a panel of cancer cell lines. Among these, compound 82 showed the greatest inhibitory activity toward HDAC 1, 3, 6 and strongly inhibited growth of the cancer cell lines, with results clearly superior to those of the reference compound, vorinostat (SAHA). Compound 82 increased the acetylation of histones H3, H4 and tubulin in a concentration-dependent manner, suggesting that it is a broad inhibitor of HDACs.

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Histone acetylation is a critical process in the regulation of chromatin structure and gene expression. In general, histone acetyltransferases (HATs) transfer acetyl groups to amino-terminal lysines in histones, which results in local expansion of chromatin and increases the accessibility of regulatory proteins to DNA, whereas histone deacetylases (HDACs) remove the acetyl groups, leading to chromatin condensation and transcriptional repression. HDAC inhibitors are considered as a new class of anticancer agents and have been shown to alter gene transcription and exert antitumor effects, such as growth arrest, differentiation, apoptosis and inhibition of tumor angiogenesis. The reported HDAC inhibitors include hydroxamates, benzamides, cyclic peptides, thiols and sulfamides, some of which have been approved for use, such as Vorinostat (SAHA), Belinostat, Panobinostat, Romidepsin and Chidamide. Among these drugs Vorinostat, Belinostat and Panobinostat are hydroxamates, indicating that this chemical group is an effective pharmacophore for HDAC inhibitors. These inhibitors generally have three characteristic structural features: a critical zinc-binding region, a hydrophobic region referred as CAP, and a linker between these two regions.

In our previous work we designed a software called CCLab (Combinatorial Chemistry Laboratory) and applied it for the identification of new chemical scaffolds of HDAC inhibitors. Based on the screening results, the scaffold of tetrahydroisoquinoline was selected for further optimization. The synthesis route is outlined in Scheme 1. The starting material phenethylamine was treated with methyl chloroformate and the intermediate product underwent cyclization to give 2. Compound 2 was nitrated by a mixture of fuming nitric acid and concentrated sulfuric acid to yield 3. Compound 4 was the reductive product of 3. Compound 4 was treated with tert-butyl nitrite and cupric bromide to give 5. Compound 5 coupled with

**Figure 1** Launched drugs targeting HDAC and the scaffold of tetrahydroisoquinoline.

**Scheme 1** Synthetic routes of HDAC inhibitors 58–83. Reagents and conditions: (a) (i) CICOOMe, Et3N, dichloromethane; (ii) polyphosphoric acid, 120 °C; (b) fuming HNO3, conc. H2SO4, 0 °C; (c) H2, Pd/C; (d) t-BuONO, CH3CN, 80 °C; (e) RBr, NaH, N,N-dimethylformamide, 80 °C; (f) methyl acrylate, Pd2(dba)3, tris(ø-tolyl)phosphine, Et3N, N,N-dimethylformamide, 120 °C; (g) NH2OH·HCl, KOH, MeOH.
different bromides to obtain 6–31, which were further modified through the Heck reaction with methyl acrylate to yield compounds 32–57. These compounds reacted with hydroxylamine in methanolic solution to afford the desired compounds 58–83.

Compound 88 was prepared following the steps in Scheme 2. 6-Methoxy-3,4-dihydroisoquinolin-1(2H)-one and benzyl bromide were treated with NaH to give compound 85, which was demethylated in the presence of BBr3 to yield compound 86. Compound 86 was treated with trifluoromethanesulfonic anhydride to give the corresponding ester which was further modified in the Heck reaction with methyl acrylate to yield compound 87. Compound 87 reacted with hydroxylamine in methanolic solution to afford the desired compound 88.

The N-hydroxycrylamide moiety can link to the tetrahydroisoquinoline ring at different positions. At the beginning we intended to investigate the impact of different positions of modification on activities (Table 1). When substituted at the 7-position, the compound (58) yielded slightly better results than the corresponding modification at the 6-position on the three tested members of HDACs (HDAC 1, 3 and 6). Thus we chose 7-position for further derivatives.

The benzyl group of 58 is hypothesized to be the CAP region. As the known HDAC inhibitors differ greatly in this moiety, we introduced different kinds of fragments to this region including substituted benzyl groups, alkyl groups and cycloalkyl groups for preliminary structure-activity relationship (SAR) information (Table 2).

All the compounds with different kinds of R groups exhibited inhibitory activity with HDAC1, 3, and 6. Longer linear alkyl groups showed a small decrease in activity (62). Cycloalkyl groups (63, 64) showed activity equivalent to that of the benzyl groups. Compounds with electron-withdrawing or electron-donating substitutions on the benzyl groups demonstrated similar activities on HDAC1, 3, and 6. However, when two methyl groups were introduced (71) the IC50 of HDAC1 was increased up to 80 nmol/L while IC50 of HDAC6 decreased to the μmol/L level. As reported, there are residues that may produce π-π stacking interactions with the CAP region. Thus we chose 58 as the hit compound to elaborate the impact of subtle modifications to the phenyl ring (Table 3).

When the phenyl ring was substituted by a pyridyl group (72), the inhibition of HDAC1 decreased markedly. When another phenyl ring or pyridyl ring was introduced to the 4-position of the benzyl group (74–76), the compounds maintained inhibitory activity, superior to that obtained with the substitution in the 2-position (77). When two more carbon atoms were introduced between the benzyl group and the tetrahydroisoquinoline ring (79),

Table 1 Inhibition on HDAC1, 3 and 6 of compound 58 and 88.

| Compd. | Structure | IC50 (μmol/L) |
|--------|-----------|--------------|
|        |           | HDAC1 | HDAC3 | HDAC6 |
| SAHA   | ![SAHA Structure](attachment:image1.png) | 0.06±0.02 | 0.09±0.02 | 0.16±0.02 |
| 58     | ![58 Structure](attachment:image2.png) | 0.22±0.05 | 0.20 | 0.18±0.03 |
| 88     | ![88 Structure](attachment:image3.png) | 0.47±0.05 | 0.37±0.04 | 2.14±0.17 |

All assay data are reported as the average of at least two measurements.
the inhibition on HDAC1, 3 and 6 increased and became equivalent to marketed drug SAHA.

As compound 79 showed activity comparable to SAHA, it was evaluated for its effect on cancer cell proliferation. Seventeen cell lines were tested. Most of the IC50 values were less than 5 μmol/L and some of them were less than 1 μmol/L. Compound 79 showed inhibition equivalent to that of SAHA (Table 4). The introduction of the phenylpropyl group enhanced the activity and showed stronger inhibition than that of SAHA, which made compound 79 a promising compound for further investigation. Several substitutions were introduced in the phenyl ring (Table 5). Substitutions in the 4-position made an obvious improvement in the inhibition of HDAC1 and 3, and the IC50 of 81 and 82 was less than 100 nmol/L. Compound 82, together with 79 and SAHA were submitted to testing on a panel of cancer cell lines for comparison (Fig. 2).

As illustrated in Fig. 2, Compound 82 strongly inhibited the proliferation of all 8 cell lines, which was clearly superior to SAHA. The introduction of a methoxy group in the phenyl ring also enhanced the activity as compared to compound 79.

One of the important functions of HDACs is to deacetylase histones and thereby suppress gene expression. As reported, SAHA increased the acetylation of histone H3, H4 and tubulin after 24 h treatment in HCT-116 colorectal cancer cells. Compound 82 also increased the acetylation of histones H3, H4 and tubulin in a concentration-dependent manner (Fig. 3), confirming that it is a pan-inhibitor of HDAC.

In summary, the scaffold of tetrahydroisoquinoline was selected from our previous work and submitted for SAR investigation. The linkage between N-hydroxyacrylamide and the tetrahydroisoquino

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**Table 2** Inhibition on HDAC1, 3 and 6 of compound 57–371.

| Compd. | R | IC50 (μmol/L) |
|--------|---|--------------|
|        |   | HDAC1       | HDAC3 | HDAC6 |
| 59     | CH₃ | 0.49         | 0.38  | 0.15  |
| 60     | C₃H₇ | 0.59        | 0.15  | 0.21  |
| 61     | C₅H₁₀ | 0.28       | 0.15  | 0.11  |
| 62     | C₁₁H₂₃ | 0.99      | 0.80  | 0.79  |
| 63     | Δ    | 0.24        | 0.22  | 0.085 |
| 64     |        | 0.13        | 0.19  | 0.14  |
| 65     | O    | 0.11        | 0.19  | 0.16  |
| 66     | CH₂   | 0.28        | 0.9   | 0.23  |
| 67     | CH₂   | 0.31        | 0.42  | 0.27  |
| 68     | CO₂   | 0.15        | 0.38  | 2.73  |
| 69     | OCF₃  | 0.25        | 0.73  | 0.21  |
| 70     | CH₂OH | 0.26        | 0.70  | 0.29  |
| 71     |        | 0.08        | 0.28  | 2.58  |

All assay data are reported as the average of at least two measurements.
line ring was better at the 7-position than the 6-position. Compounds with a phenylpropyl group in the CAP region exhibited good activities on HDAC1, 3 and 6, and further modification of the phenyl group gave compound 82, which showed excellent HDAC inhibition and strong inhibition of proliferation of several cancer cell lines.

### Table 3 Inhibition on HDAC1, 3 and 6 of compound 72–79.

| Compd. | IC$_{50}$ (μmol/L) | HDAC1 | HDAC3 | HDAC6 |
|--------|---------------------|-------|-------|-------|
| 72     | 1.41                | 0.43  | 0.42  |
| 73     | 0.47                | 0.30  | 0.30  |
| 74     | 0.19                | 0.14  | 0.26  |
| 75     | 0.15                | 0.22  | 3.91  |
| 76     | 0.13                | 0.31  | 0.14  |
| 77     | 0.54                | 0.84  | 3.21  |
| 78     | 0.25                | 0.81  | 0.196 |
| 79     | 0.14                | 0.06  | 0.08  |

All assay data are reported as the average of at least two measurements.

### Table 4 IC$_{50}$ values of compound 79 and SAHA in cancer cell lines (μmol/L).

| Cell lines | SAHA     | 79       |
|------------|----------|----------|
| HeLa       | 3.62 ± 0.27 | 2.53 ± 0.75 |
| BEL-7402   | 3.01 ± 0.53 | 1.50 ± 0.09 |
| SMMC-7721  | 2.68 ± 1.03 | 2.76 ± 1.32 |
| SGC-7901   | 2.84 ± 1.67 | 2.38 ± 0.61 |
| MKN28      | 2.21 ± 0.84 | 1.40 ± 0.55 |
| A549       | 3.79 ± 1.98 | 2.28 ± 1.00 |
| MCF-7      | 2.08 ± 1.19 | 0.93 ± 0.31 |
| MDA-MB-468 | 2.98 ± 1.25 | 1.57 ± 0.96 |
| PC3        | 5.43 ± 1.78 | 1.94 ± 0.24 |
| U251       | 10.08 ± 1.45 | 7.68 ± 2.31 |
| A431       | 2.07 ± 0.13 | 2.31 ± 0.41 |
| A375       | 1.86 ± 0.42 | 2.09 ± 1.20 |
| T24        | 2.18 ± 1.09 | 4.01 ± 0.90 |
| SK-OV-3    | 3.57 ± 0.74 | 1.04 ± 0.38 |
| BxPC3      | 2.24 ± 0.61 | 1.04 ± 0.38 |
| 786-O      | 4.01 ± 1.30 | 3.83 ± 2.18 |
| GES-1      | 1.12 ± 0.11 | 0.57 ± 0.23 |
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References

1. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Annu Rev Biochem 2001;70:81–120.
2. Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JP. Histone deacetylases: unique players in shaping the epigenetic histone code. Ann NY Acad Sci 2003;983:84–100.
3. Marks PA. Discovery and development of SAHA as an anticancer agent. Oncogene 2007;26:1351–6.
4. Molife LR, De Bono JS. Belinostat: clinical applications in solid tumors and lymphoma. Expert Opin Investig Drug 2011;20:1723–32.
5. Richardson PG, Laubach JP, Moreau P, Yoon SS, Hungria VTM, et al. Panobinostat: a novel pan-deacetylase inhibitor for the treatment of relapsed or relapsed and refractory multiple myeloma. Expert Rev Anticancer Ther 2015;15:737–48.
6. Slingerland M, Guchelaar HJ, Gelderblom H. Histone deacetylase inhibitors. Anti-Cancer Drugs 2014;25:140–9.
7. Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014;13:673–91.
8. Fang GH, Xue MZ, Su MB, Hu DY, Li YL, Xiong B, et al. CCLab—a multi-objective genetic algorithm based combinatorial library design software and an application for histone deacetylase inhibitor design. Bioorg Med Chem Lett 2012;22:4540–5.
9. Compound 82: 1H NMR (400 MHz, CD3OD) δ 8.12 (s, 1H), 7.63–7.57 (m, 2H), 7.30 (m, 1H), 7.64 (s, 1H), 7.13 (d, J=8.4 Hz, 2H), 6.81–6.79 (m, 2H), 6.51 (d, J=16.0 Hz, 1H), 3.73 (s, 3H), 3.62–3.56 (m, 4H), 2.98 (t, J=6.4 Hz, 2H), 2.62 (t, J=7.6 Hz, 2H), 1.95–1.92 (m, 2H); LRMS (ESI) m/z 381.2 [M+H]+ HRMS Calcd. for C22H25N2O4: 381.1809 [M+H]+; Found: 381.1809.
10. Shen J, Woodward R, Kedendurg JP, Liu XW, Chen M, Fang LY, et al. Histone deacetylase inhibitors through click chemistry. J Med Chem 2008;51:7417–27.

Table 5  Inhibition on HDAC1, 3 and 6 of compound 80–83.

| Compd. | R | IC₅₀ (µmol/L) |
|--------|---|-------------|
|        |   | HDAC1 | HDAC3 | HDAC6 |
| 80     |   | 0.23  | 0.30  | 3.51  |
| 81     |   | 0.03  | 0.03  | 0.14  |
| 82     |   | 0.03  | 0.07  | 0.18  |
| 83     |   | 0.10  | 0.10  | 0.12  |

All assay data are reported as the average of at least two measurements.

Figure 2  IC₅₀ values of compound 79, 82 and SAHA on cancer cell lines (µmol/L).

Figure 3  Increased acetylated histones and tubulin levels in compound 82 and SAHA treated HCT-116 colorectal cancer cells. HCT-116 cells were treated with various concentrations of compound 82 and SAHA for 24 h, proteins extracted, and analyzed for Ac-H3, Ac-H4 and Ac-tubulin by Western Blot.14
11. Salvador LA, Luessch H. Discovery and mechanism of natural products as modulators of histone acetylation. *Curr Drug Targets* 2012;13:1029–47.

12. Wang D. Computational studies on the histone deacetylases and the design of selective histone deacetylase inhibitors. *Curr Top Med Chem* 2009;9:241–56.

13. Cell proliferation assay: Cells were seeded in 96-well culture plates. After incubation overnight, cells were exposed to the indicated concentrations of the compounds for a further 72 h. Cells were then fixed with 10% pre-cooled trichloroacetic acid, washed with distilled water, and stained with 4 mg/mL SRB (Sigma) in 1% acetic acid. Protein-bound SRB in the cells was dissolved in 10 mmol/L Tris-HCl and was measured at 560 nm using spectraMax190 (Molecular Devices, CA, USA). The IC$_{50}$ values were calculated by concentration-response curve-fitting using the SoftMax pro-based four-parameter method and shown as the mean±SD from two separate experiments.

14. Western Blot analysis: HCT-116 colorectal cancer cells were exposed to various concentrations of the compounds for 24 h at 37 °C. The cells were treated with the indicated concentration of the selected compounds for 24 h at 37 °C and then lysed in 1× SDS sample buffer. Cell lysates were resolved on 12% SDS-PAGE and transferred to nitrocellulose membranes. The transfers were incubated with specific primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies. The immunoreactive proteins were detected using an ECL plus detection reagent (Pierce, Rockford, IL, USA) and imaged by autoradiography.