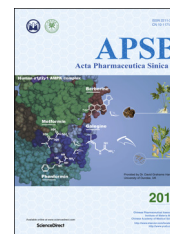




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SHORT COMMUNICATION

# Tetrahydroisoquinolines as novel histone deacetylase inhibitors for treatment of cancer



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## KEY WORDS

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inhibitor;  
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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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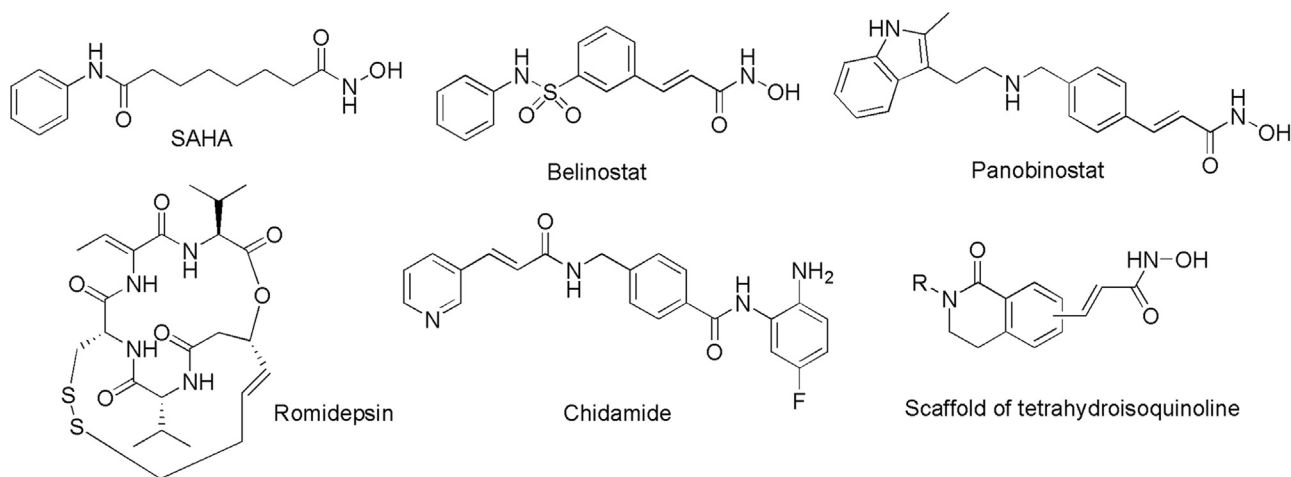
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Histone acetylation is a critical process in the regulation of chromatin structure and gene expression. In general, histone acetyltransferases (HATs) transfer acetyl groups to amino-terminal lysines in histones, which results in local expansion of 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<sup>1,2</sup>. 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 sulphamides, some of which have been approved for use, such as Vorinostat (SAHA)<sup>3</sup>, Belinostat<sup>4</sup>, Panobinostat<sup>5</sup>, Romidepsin<sup>6</sup> and Chidamide (Fig. 1). Among these drugs Vorinostat, Belinostat and Panobinostat are hydroxamates, indicating that

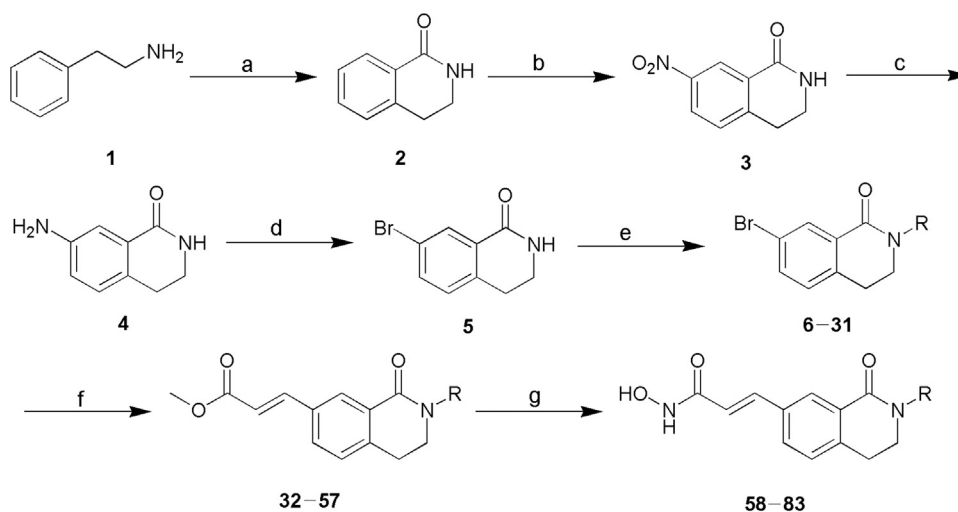
this chemical group is an effective pharmacophore for HDAC inhibitors. These inhibitors generally have three characteristic structural features: a critical zinc-binding region, a hydrophobic region referred as CAP, and a linker between these two regions<sup>7</sup>.

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<sup>8</sup>. Based on the screening results, the scaffold of tetrahydroisoquinoline (Fig. 1) was selected for further optimization.

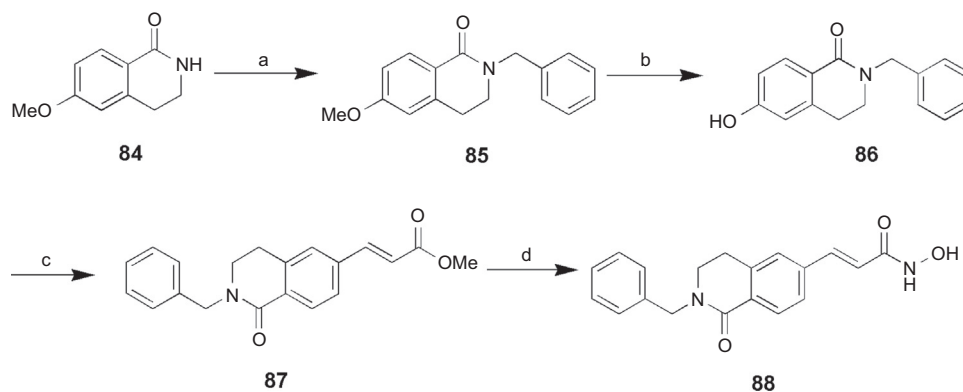
The synthesis route is outlined in Scheme 1. The starting material phenethylamine was treated with methyl chloroformate and the intermediate product underwent cyclization to give **2**. Compound **2** was nitrated by a mixture of fuming nitric acid and concentrated sulfuric acid to yield **3**. Compound **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) ClCOOMe, Et<sub>3</sub>N, dichloromethane; (ii) polyphosphoric acid, 120 °C; (b) fuming HNO<sub>3</sub>, conc. H<sub>2</sub>SO<sub>4</sub>, 0 °C; (c) H<sub>2</sub>, Pd/C; (d) *t*-BuONO, CH<sub>3</sub>CN, 80 °C; (e) RBr, NaH, *N,N*-dimethylformamide, 80 °C; (f) methyl acrylate, Pd<sub>2</sub>(dba)<sub>3</sub>, tris(*o*-tolyl)phosphine, Et<sub>3</sub>N, *N,N*-dimethylformamide, 120 °C; (g) NH<sub>2</sub>OH·HCl, KOH, MeOH.



**Scheme 2** Synthetic procedure of compound **88**. Reagents and conditions: (a) NaH, benzyl bromide, *N,N*-dimethylformamide, 100 °C; (b) BBr<sub>3</sub>, dichloromethane, r.t.; (c) (i) trifluoromethanesulfonic anhydride, dichloromethane, 0 °C to r.t.; (ii) methyl acrylate, Pd<sub>2</sub>(dba)<sub>3</sub>, tris(*o*-tolyl)-phosphine, Et<sub>3</sub>N, *N,N*-dimethylformamide, 120 °C; (d) NH<sub>2</sub>OH · HCl, KOH, MeOH.

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

Compd.	Structure	IC <sub>50</sub> (μmol/L)		
		HDAC1	HDAC3	HDAC6
SAHA		0.06±0.02	0.09±0.02	0.16±0.02
<b>58</b>		0.22±0.05	0.20	0.18±0.03
<b>88</b>		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.

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**<sup>9</sup>.

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 BBr<sub>3</sub> 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**.

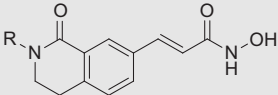
The *N*-hydroxyacrylamide moiety can link to the tetrahydroisoquinoline ring at different positions. At the beginning we intended to investigate the impact of different positions of modification on activities ([Table 1](#)). When substituted at the 7-position, the compound (**58**) yielded slightly better 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 IC<sub>50</sub> of HDAC1 was increased up to 80 nmol/L while IC<sub>50</sub> of HDAC6 decreased to the μmol/L level. As reported, there are residues that may produce  $\pi$ - $\pi$  stacking interactions with the CAP region<sup>10–12</sup>. 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 2** Inhibition on HDAC1, 3 and 6 of compound **57–371**.


Compd.	R	IC <sub>50</sub> (μmol/L)		
		HDAC1	HDAC3	HDAC6
<b>59</b>	*-CH <sub>3</sub>	0.49	0.38	0.15
<b>60</b>	*-C <sub>3</sub> H <sub>7</sub>	0.59	0.15	0.21
<b>61</b>	*-C <sub>6</sub> H <sub>13</sub>	0.28	0.15	0.11
<b>62</b>	*-C <sub>11</sub> H <sub>23</sub>	0.99	0.80	0.79
<b>63</b>	*-Cyclopropyl	0.24	0.22	0.085
<b>64</b>	*-Cyclohexyl	0.13	0.19	0.14
<b>65</b>	*-4-Methoxyphenyl	0.11	0.19	0.16
<b>66</b>	*-4-Methylphenyl	0.28	0.9	0.23
<b>67</b>	*-4-Tert-butylphenyl	0.31	0.42	0.27
<b>68</b>	*-4-Toluenesulfonylphenyl	0.15	0.38	2.73
<b>69</b>	*-4-(Trifluoromethoxy)phenyl	0.25	0.73	0.21
<b>70</b>	*-4-(Hydroxylamino)phenyl	0.26	0.70	0.29
<b>71</b>	*-3,4-Dimethylphenyl	0.08	0.28	2.58

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 IC<sub>50</sub> 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)<sup>13</sup>.

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 IC<sub>50</sub> 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 deacetylate 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

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

Compd.	R	IC <sub>50</sub> (μmol/L)		
		HDAC1	HDAC3	HDAC6
<b>72</b>		1.41	0.43	0.42
<b>73</b>		0.47	0.30	0.30
<b>74</b>		0.19	0.14	0.26
<b>75</b>		0.15	0.22	3.91
<b>76</b>		0.13	0.31	0.14
<b>77</b>		0.54	0.84	3.21
<b>78</b>		0.25	0.81	0.196
<b>79</b>		0.14	0.06	0.08

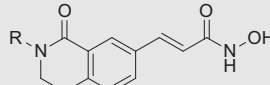
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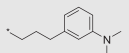
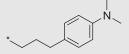
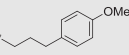
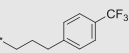
**Table 4** IC<sub>50</sub> values of compound **79** and SAHA in cancer cell lines (μmol/L).

Cell lines	SAHA	<b>79</b>
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 ± 0.317
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

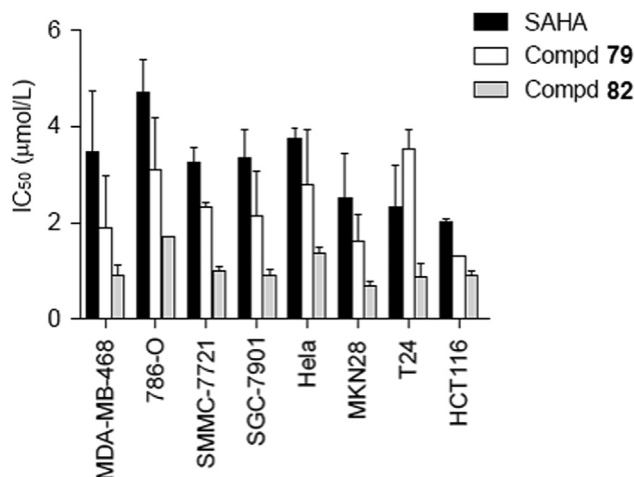
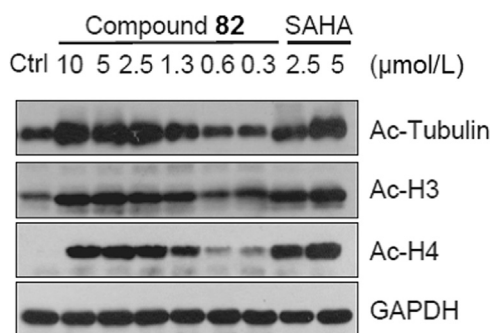
line ring was better at the 7-position than the 6-position. Compounds with a phenylpropyl group in the CAP region exhibited good activities on HDAC1, 3 and 6, and further

modification of the phenyl group gave compound **82**, which showed excellent HDAC inhibition and strong inhibition of proliferation of several cancer cell lines.

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


Compd.	R	IC <sub>50</sub> (μmol/L)		
		HDAC1	HDAC3	HDAC6
<b>80</b>		0.23	0.30	3.51
<b>81</b>		0.03	0.03	0.14
<b>82</b>		0.03	0.07	0.18
<b>83</b>		0.10	0.10	0.12

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

**Figure 2** IC<sub>50</sub> 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<sup>14</sup>.

### Acknowledgments

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- Compound **82**: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.12 (s, 1H), 7.63–7.57 (m, 2H), 7.30 (d, *J*=7.6 Hz, 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]<sup>+</sup> HRMS Calcd. for C<sub>22</sub>H<sub>25</sub>N<sub>2</sub>O<sub>4</sub>: 381.1809 [M+H]<sup>+</sup>; Found: 381.1809.
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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<sub>50</sub> 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.