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Studying Histone Deacetylase Inhibition and Apoptosis Induction of Psammaplin A Monomers with Modified Thiol Group

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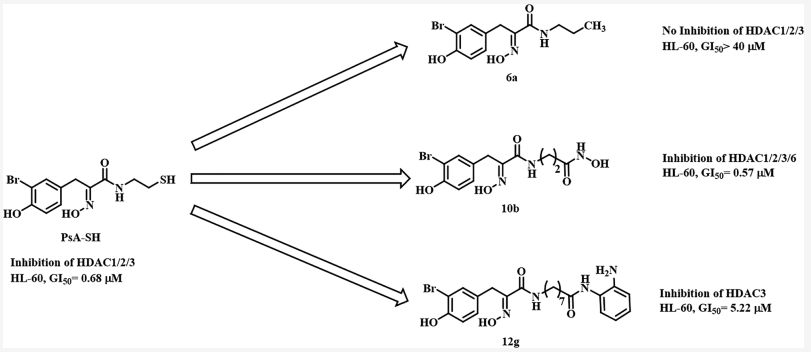

ABSTRACT: Psammaplin A (PsA) is a bromotyrosine disulfide dimer with histone deacetylase (HDAC) inhibition and acts through reduced monomer PsA-SH. We studied the connection of HDAC inhibition, cell growth inhibition, and apoptosis induction of PsA-SH by modifying the −SH group with deletion (**6a**) or replacement with hydroxamic acid (**10b**) or benzamide (**12g**). PsA-SH inhibits HDAC1/2/3 and **6a** loses the HDAC inhibition ability. **10b** inhibits HDAC1/2/3/6 while **12g** shows selective inhibition of HDAC3. PsA-SH and **10b**, but neither **6a** nor **12g**, induce apoptosis in human leukemia HL-60 cells associated with increased acetylation of Histone H3. PsA-SH and **10b** inhibit growth of several solid tumor cell lines in vitro and Lewis lung cancer cell growth in vivo. PsA-SH is a simple scaffold for developing selective HDAC inhibitors and induces apoptosis through inhibiting HDAC1/2.

KEYWORDS: *Psammaplin A, HDAC, sulfydryl, hydroxamic acid, benzamide*

Chromatin is a dense and higher-order structure consisting of DNA and core histone proteins. Histone deacetylases (HDACs) catalyze deacetylation of the N-terminal lysine residues of the core histones.[1] Deacetylation of histones causes chromatin aggregation which prevents transcriptional machinery to be close to DNA and represses the gene transcription.[2] Aberrant acetylation of histone and/or expression of HDACs occur in cancer cells and contribute to tumorigenesis.[3] Eighteen HDAC subtypes have been found in mammalian cells and are divided into four classes. Classes I, II, and IV are Zn^{2+}-dependent HDACs, while class III is NAD^{+}-dependent sirtuins.[4,5] Class I HDACs contain HDAC-1, -2, -3, and -8. Class II HDACs are further divided into class IIa (HDAC-4, -5, -7, -9) and class IIb (HDAC-6, -10). Overexpression of class I HDAC isotypes occurs in aggressive subtypes of tumors such as stomach, esophagus, colorectal, and breast cancers.[6−11] Genetic knockdown of individual HDAC, most notably HDAC-1, -2, and -3, induced DNA damage, cell cycle arrest, and apoptosis.[6] HDACs, especially class I, have been used as targets for developing cancer therapeutics.

A large group of small-molecules have been developed as HDAC inhibitors for cancer therapy in the last two decades.[12,13] Vorinostat (SAHA), Romidepsin (FK-228),[14] Belinostat (PXD-101), and Panobinostat (LBH-589)[15] have been approved by the USA FDA and Chidamide (CS055/HBI-8000)[16] by the China FDA for T-cell lymphoma and myeloma therapies (Figure 1). These inhibitors have not been extended to other types of cancer therapy probably due to the obvious side effects and/or unconfirmed effectiveness.[17−19] More selective inhibitors need to be developed for decreasing the side effects and/or for increasing the efficacies. HDAC inhibitors usually contain three portions: zinc binding group (ZBG), linker region, and surface recognition moiety (SRM).

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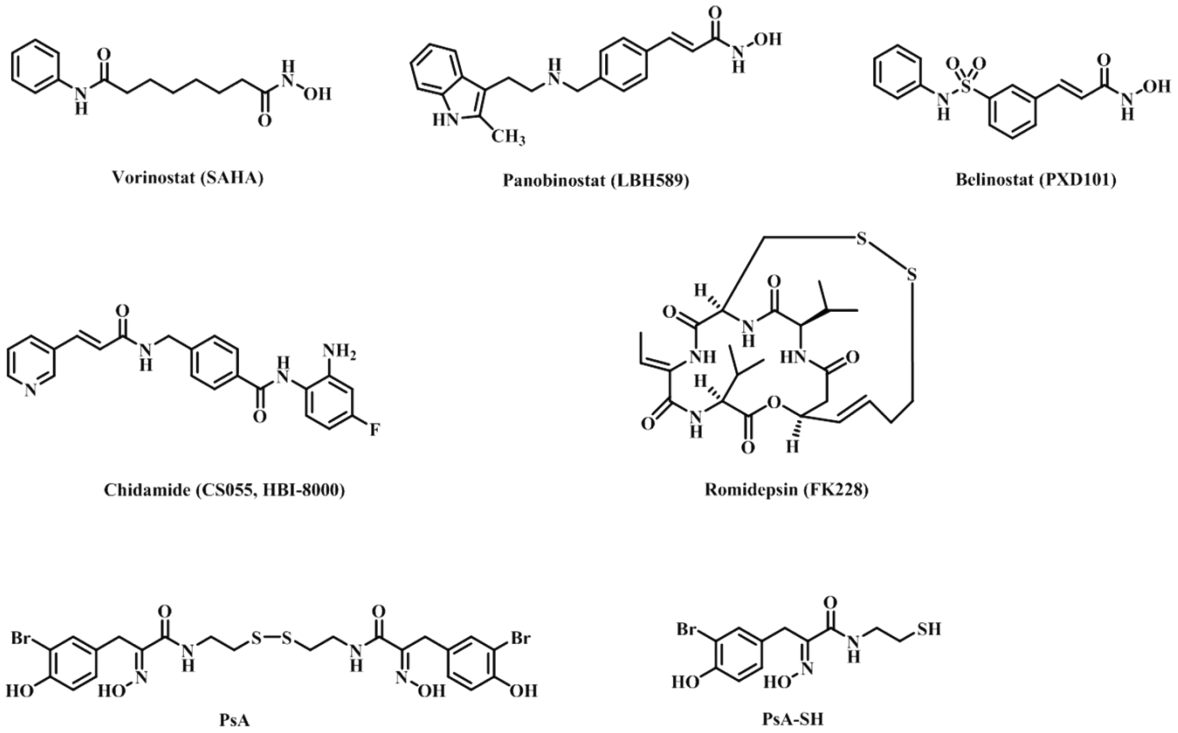
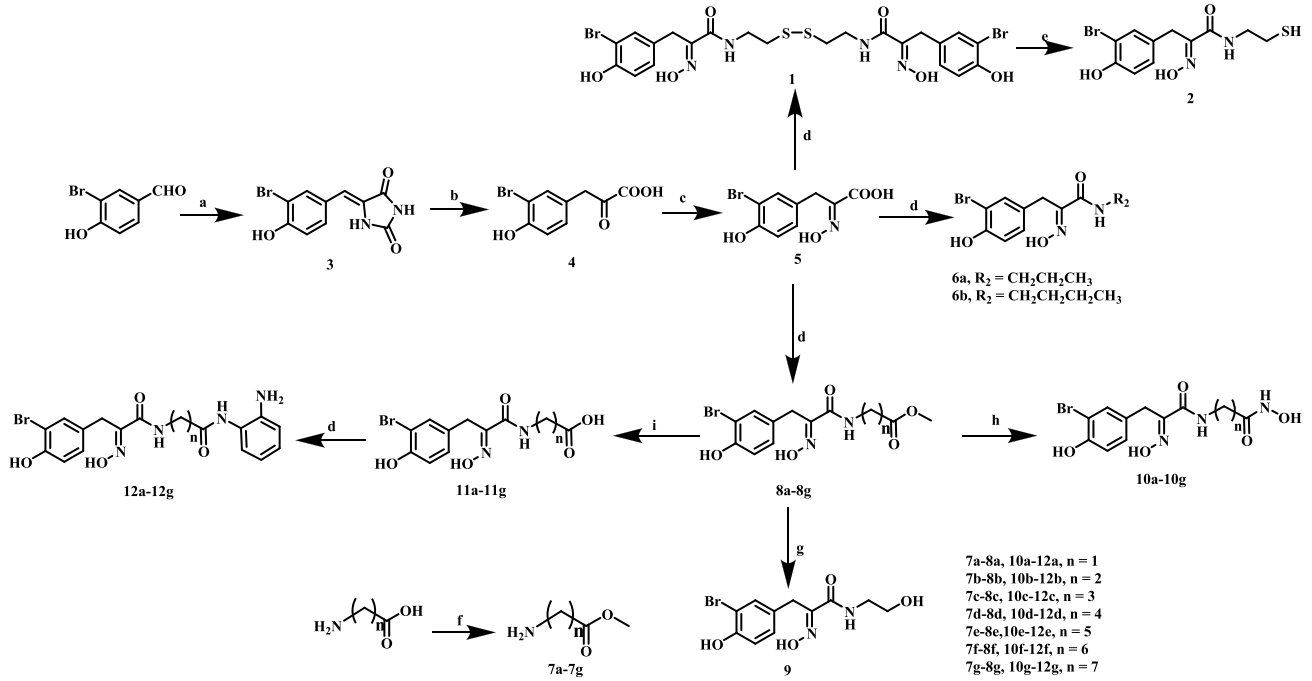


Figure 1. Chemical structures of Psammaplin A (PsA), Psammaplin A monomer (PsA-SH), and approved HDAC inhibitors.

Scheme 1. Synthesis of PsA Monomer with a Different ZBG[a]



[a]Reagents and conditions: (a) Imidazolidine-2,4-dione, $NaHCO_3$, H_2O, 120 °C, 10 h, 83−92%; (b) NaOH, N_2, H_2O, 95 °C, 12 h, 65−73%; (c) NaOH, $NaHCO_3$, $HONH_2$·HCl, H_2O, r.t., 12 h, 70−84%; (d) HOBt, EDCI, DIEA, DMF, 10 h, 30−47%; (e) DTT, KOH, MeOH, r.t., 30 min; (f) $SOCl_2$, MeOH, Et_2O, r.t., 3 h, 74−83%; (g) $LiAlH_4$, THF, r.t., 1 h; (h) $HONH_2$·H_2O, NaOH, MeOH, r.t., 30 min, 73−88%; (i) NaOH, MeOH, 60 °C, 30 min, 90−95%.

The ZBG plays an important role in determining the selectivity of HDAC inhibitors. Vorinostat, Belinostat, and Panobinostat containing the hydroxamic acid are pan-HDAC inhibitors. Chidamide containing the benzamide is a relative Class I HDAC inhibitor. A great effort of developing HDAC inhibitors has been focused on compounds with either hydroxamic acid or benzamide as the ZBG.[12,13] Although many new HDAC inhibitors are put in clinical trials, there is none approved yet.[20] HDAC inhibitors with a novel structure need to be considered. Romidepsin is a natural product derived from *Chromobacte-*

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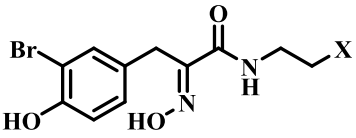
Table 1. Activities of PsA Monomers with the Modified −SH to Inhibit HDAC Activity and Cell Growth



Compd.	X-Group	HL-60 GI_{50} [μM][a]	HDAC1 IC_{50} [μM]	HDAC2 IC_{50} [μM]	HDAC3 IC_{50} [μM]	HDAC6 IC_{50} [μM]
PsA[b]	−S-Dimer	0.37 ± 0.04	0.008 ± 0.006	0.033 ± 0.007	0.0124 ± 0.0004	0.311 ± 0.008
PsA-SH	−SH	0.68 ± 0.08	0.052 ± 0.018	0.21 ± 0.16	0.12 ± 0.02	0.96 ± 0.05
6a	$-CH_3$	13.5 ± 0.5	>10	>10	>10	>10
6b	$-CH_2CH_3$	36 ± 4	>10	>10	>10	>10
9	−OH	20 ± 2	>10	>10	>10	>10
11b	−COOH	11.8 ± 1.2	>10	>10	>10	>10

[a]HL-60 cells were treated for 72 h. Values are means plus SD of three independent experiments. [b]PsA was reduced with TCEP before enzyme activity inhibition assay.

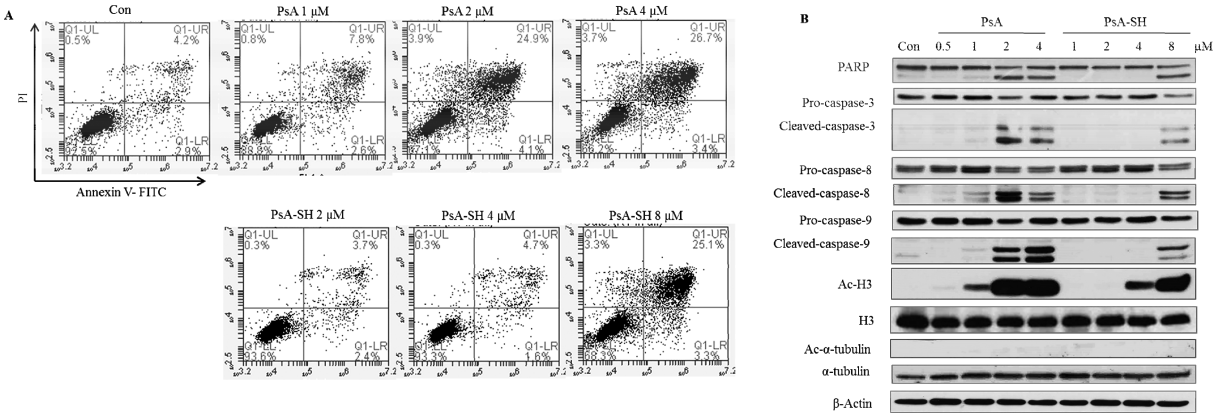


Figure 2. FACS and Western blot analyses of apoptosis induction by PsA and PsA-SH in HL-60 cells. (A) HL-60 cells were treated with PsA and PsA-SH at the indicated concentrations for 24 h, and apoptotic cells were determined by FACS after staining with PI/Annexin-V. (B) Western blot analysis of the protein levels of Ac-H3, H3, Ac-α-tubulin, α-tubulin, PARP, caspase-3, -8, -9, and β-actin of HL-60 cells treated with PsA and PsA-SH for 24 h.

rium violaceum with a cyclic peptide containing a disulfide bond (Figure 1). Romidepsin is a class I selective HDAC inhibitor and needs the reduced thiol group (−SH) to coordinate the Zn^{2+} for HDAC inhibition.[21] Compounds with a disulfide bond and/or thiol group would be a great source of developing Class I HDAC selective inhibitors.

Psammaplin A (PsA) is a symmetrical bromotyrosine-derived disulfide dimer isolated from the *Psammaplysilla sponge* (Figure 1). PsA has various bioactivities such as antimicrobial activity and cytotoxicity against cancer.[22] PsA has been shown to inhibit several enzymes including DNA gyrase, DNA topoisomerase, farnesyl protein transferase, and leucine aminopeptidase.[22] More intriguingly, PsA was found to be a potent inhibitor of HDAC.[23,24] Further studies revealed that PsA, like Romidepsin, is a Class I HDAC inhibitor[25] and needs to be reduced into PsA-SH monomer for HDAC inhibition.[24,26] Previous modifications of PsA have been focused on the bromotyrosine moiety with the dimer structure.[24,25,27,28] PsA inhibited cancer cell growth with apoptosis induction, but the connection with HDAC inhibition was not clearly understood.[25,29−31] We take the advantage of the simple structure of PsA-SH to dissect connection of HDAC inhibition and apoptosis induction by modifying the −SH group. We designed and synthesized PsA-SH monomer analogues with deleted −SH or replaced by the hydroxamic acid or by the benzamide, and compared their growth inhibition and apoptosis induction in tumor cells.

We generated three groups of compounds without changing the SRM of PsA-SH. The synthetic routes to compounds PsA (**1**), PsA-SH (**2**), **6a−6b**, **9**, **10a−10g**, **11b**, and **12a−12g** are depicted in Scheme 1. Coupling of 3-bromo-4-hydroxybenzaldehyde with imidazolidine-2,4-dione gave **3**, which was then hydrolyzed in NaOH aqueous to afford **4**, which was subsequently treated with hydroxylamine hydrochloride and $NaHCO_3$ under basic condition to afford **5**. The intermediate **5** was treated with cystamine dihydrochloride in DMF to afford compound **1**, which was subsequently treated with *DL*-dithiothreitol under basic condition to afford **2**. Coupling of intermediate **5** with appropriate amine gave the compounds **6a−6b**. The appropriate amino acids were treated with $SOCl_2$ in methanol to afford intermediates **7a−7g**, then **8a−8g** were prepared by coupling the above methyl ester with intermediate **5**. The intermediate **8a** was reduced with $LiAlH_4$ to afford compound **9**. The intermediates **8a−8g** were treated with 50% hydroxylamine under basic conditions to afford compounds **10a−10g**. The intermediates **8a−8g** were hydrolyzed in aqueous NaOH to afford intermediates **11a−11g**. Then coupling of **11a−11g** with benzene-1,2-diamine gave compounds **12a−12g**.

The first group of compounds are PsA-SH with deleted −SH. We kept the scaffold of PsA-SH and converted the −SH to −Me, −Et, −OH, and −COOH, respectively, and obtained analogues **6a**, **6b**, **9**, and **11b**. The inhibitory effects of these compounds on HDAC activity and cell growth were tested

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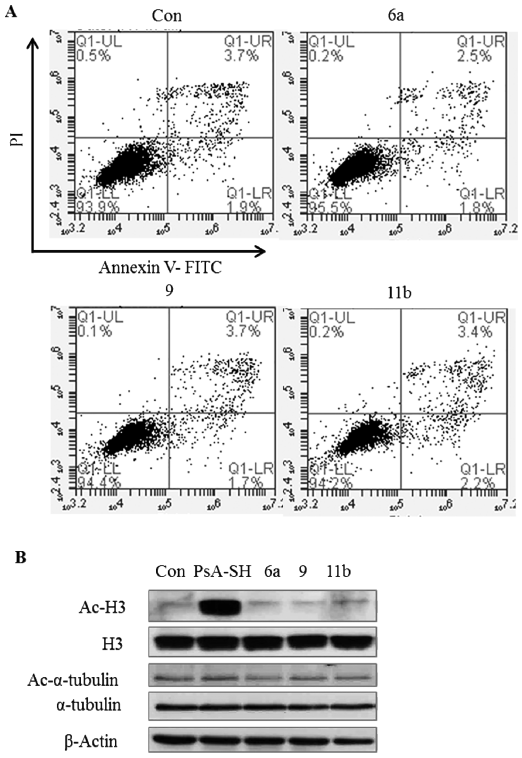


Figure 3. Apoptosis and H3 acetylation levels of HL-60 cells treated with **6a**, **9**, and **11b**. Apoptotic cells were determined by FACS after staining with PI/Annexin-V (A), and the acetylated levels of H3 and α-tubulin were measured with Western blot (B) in HL-60 cells treated with each compound at 20 μM for 24 h.

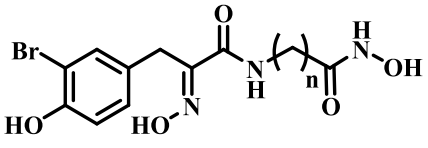
using specific recombinant HDAC enzymes and leukemia HL-60 cells and were compared with that of PsA (**1**) and PsA-SH (**2**). It is to be noted that PsA was reduced with tris(2-carboxyethyl)phosphine (TCEP) before determining HDAC activity inhibition as reported.[25] PsA inhibited activities of HDAC1, 2, and 3 with an IC_{50} of 8 nM, 33 nM, and 12.4 nM, respectively, and inhibited HDAC6 with an IC_{50} of 311 nM (Table 1). PsA-SH has similar inhibitory effects on HDAC1, 2, and 3, with a little decreased ability of inhibiting HDAC6. PsA inhibited HL-60 cell growth with a IG_{50} of 0.37 μM. PsA-SH has a IG_{50} of 0.68 μM, 2-fold higher than that of PsA, suggesting that PsA splits into two molecules of PsA-SH in cells to act. Compounds **6a**, **6b**, **9**, and **11b** did not exhibit inhibitory effects on cell growth nor on HDAC activity at a concentration up to 10 μM (Table 1). These data support that PsA converts to PsA-SH in cells and that −SH is a key functional group for inhibiting cell growth and HDAC activity.

Apoptosis induction is a key mechanism of HDAC inhibitors to inhibit tumor cell growth.[32] We tested and compared the apoptosis induction abilities of PsA and PsA-SH by measuring the content of cells stained positive for Annexin V with flow cytometry in HL-60 cells. PsA at 4 μM and PsA-SH at 8 μM induced about 30% of cells undergoing apoptosis (Figure 2A). Western blot analysis revealed that treatment with PsA and PsA-SH induced the typical apoptotic signaling changes including cleavage of PARP, caspase-3, caspase-8, and caspase-9. Both PsA and PsA-SH increased the levels of acetylated-Histone 3 (H3), the substrate of HDAC1/2/3. Although both PsA and PsA-SH exhibited an inhibitory effect on HDAC6 activity in enzyme activity assay, they did not increase the levels of acetylated α-tubulin, the specific substrate of HDAC6 (Figure 2B), suggesting that intracellular HDAC6 activity was not inhibited in cells. Compounds **6a**, **9**, and **11b** neither induced Annexin-V positive cells nor increased the levels of acetylated−H3 even at a concentration up to 20 μM (Figure 3). These data indicate that apoptosis induction by PsA and PsA-SH associates with inhibition of intracellular HDAC1, 2, and 3, but not HDAC6.

The second group of compounds are the −SH of PsA-SH converted to the hydroxamic acid. We generated compounds **10a−g** substituted with the hydroxamic acid. These compounds keep the SRM and have different linker lengths (Table 2). **10b** is a compound with direct replacement of −SH with the hydroxamic acid. **10b** maintains the abilities of inhibiting HDAC2 and 3 but with increased abilities of inhibiting HDAC1 and HDAC6, that is consistent with a previously reported work.[25] The growth inhibitory effect of **10b** in HL-60 cells is similar to that of PsA-SH. **10a** has one-carbon short linker compared to **10b** and the inhibitory effects on HDAC1, 2, 3, and 6 activity as well as on HL-60 cell growth are significantly decreased. Compounds **10c−d** have one and two carbons increased linker (Table 2). **10c** only exhibits weak inhibition on HDAC1 with IC_{50} about 1 μM. **10d** exhibits inhibition on HDAC1, 3, and 6, with an IC_{50} of 0.436 μM, 0.249 μM, and 1.29 μM, respectively. The IG_{50} of **10a**, **10c**, and **10d** of inhibiting HL-60 cell growth are higher than 40 μM. These data indicate that directly converting −SH of PsA-SH to the hydroxamic acid becomes a pan-HDAC inhibitor.

By comparing the apoptosis induction with PsA and PsA-SH it was found that **10b** was as effective as PsA to induce apoptosis in HL-60 cells (Figure 4A). Two μM of **10b** induced about 30% of cells undergoing apoptosis. Western blot analysis showed that **10b** induced cleavage of PARP and caspases with

Table 2. Inhibitory Effects of PsA Monomers with the Hydroxamic Acid Substitution on HDAC Activity and Cell Growth



Compd.	n	HL-60[a] IG_{50} [μM]	HDAC1 IC_{50} [μM]	HDAC2 IC_{50} [μM]	HDAC3 IC_{50} [μM]	HDAC6 IC_{50} [μM]
10a	1	>40	2.93	9.21	1.23	>10
10b	2	0.57 ± 0.19	0.018 ± 0.004	0.033 ± 0.001	0.020 ± 0.002	0.096 ± 0.013
10c	3	>40	1.01	>10	>10	>10
10d	4	>40	0.436	>10	0.249	1.29

[a]HL-60 cells were treated for 72 h. Values are means plus SD of three independent experiments.

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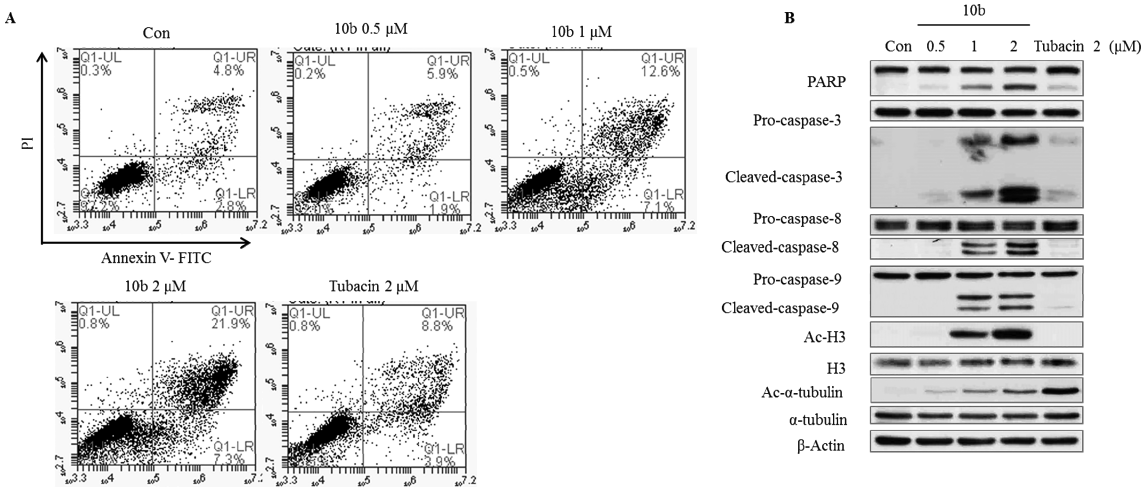
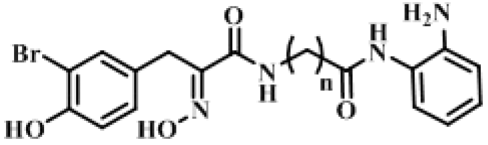


Figure 4. Apoptosis induction and protein regulation by **10b** and Tubacin. Apoptotic cells were determined by FACS after staining with PI/Annexin-V (A), and the protein levels of Ac-H3, Ac-α-tubulin, PARP, caspase-3, -8, -9, and β-actin were determined by Western blotting (B) in HL-60 cells treated with **10b** and Tubacin at the indicated concentrations for 24 h.

Table 3. Inhibitory Effects of PsA Monomers with the Benzamide Substitution on HDAC Activity and Cell Growth



Compd.	n	HL-60 IG_{50} [μM][a]	HDAC1 IC_{50} [μM]	HDAC2 IC_{50} [μM]	HDAC3 IC_{50} [μM]	HDAC6 IC_{50} [μM]
12a	1	>40	>10	>10	>10	>10
12b	2	>40	>10	>10	>10	>10
12c	3	>40	>10	>10	1.83	>10
12d	4	>40	>10	9.76	1.43	>10
12e	5	14.3 ± 3.1	>1	>1	0.18 ± 0.02	>1
12f	6	14.0 ± 3.2	>1	>1	0.13 ± 0.04	>1
12g	7	5.2 ± 0.4	>1	>1	0.17 ± 0.02	>1
RGFP966		6.1 ± 1.0	>1	>1	0.19 ± 0.05	>1

[a]HL-60 cells were treated for 72 h. Values shown are means plus SD of three independent experiments.

increased levels of both acetylated H3 and acetylated α-tubulin (Figure 4B). If looking at the dose-dependent effects of **10b** treatment, **10b** increased the levels of acetylated α-tubulin at 0.5 μM without inducing PARP cleavage and apoptosis. The HDAC6 inhibitor Tubacin increased the levels of acetylated α-tubulin without apoptosis induction. These data indicate that **10b** induces apoptosis in a similar way as PsA through inhibiting class I HDAC1/2/3 but not HDAC6.

The third group of compounds are the −SH of PsA-SH converted to the benzamide. Benzamide is used as a ZBG to develop selective Class I HDAC inhibitors.[33,34] We replaced the −SH of PSA-SH with the benzamide and generated several new analogues **12a−g**, which have a linker length of 1 to 7 carbons, respectively (Table 3). **12a** and **12b** do not show inhibitory effects on HDAC activity and cell growth. **12c−12d** have weaker inhibitory effects on HDAC3 with IC_{50} of 1.83 μM and 1.43 μM, respectively. Both compounds do not inhibit cell growth of HL-60 cells. **12e−g** have similar inhibitory effects on HDAC3 with that of PsA-SH but do not inhibit HDAC1 and HDAC2 at a concentration of 1 μM. **12e**, **12f**, and **12g** inhibited growth of HL-60 cells with an IG_{50} of 14.3 μM, 14.0 μM, and 5.2 μM, respectively, which were much higher than that of PsA-SH. RGFP966 is a selective HDAC3 inhibitor used as a tool compound. The inhibitory effects on HDAC activity and cell growth of RGFP966 and **12g** were compared (Table 3). Both compounds exhibited similar abilities of inhibiting HDAC3 activity and HL-60 cell growth. **12e−g** could be considered as novel selective HDAC3 inhibitors with new structures.

The apoptosis induction abilities of **12e**, **12f**, and RGFP966 in HL-60 cells were measured and compared. These compounds did not induce apoptosis at 20 μM. When the concentrations were increased to 40 μM, a small amount of apoptotic cells were detected (Figure 5A). Western blot analysis showed that **12e** and **12f** at 20 μM did not induce cleavage of PARP and caspases, but with induction of H3 acetylation (Figure 5B). The upregulated H3 acetylation may come from HDAC3 inhibition, which needs to be further confirmed. **12e**, **12f**, and RGFP966 do not increase the levels of acetylated H3 at 2 μM (data not shown). Comparing to PsA and **10b**, these compounds keep the ability of inhibiting HDAC3, but not HDAC1/2. Therefore, the apoptosis induction abilities of PsA, PsA-SH, and **10b** should come from their inhibition of HDAC1/2 but not HDAC3.

PsA-SH inhibits HDAC1 and HDAC3 at the same level (Table 1), while **12g** is more effective to inhibit HDAC3 than HDAC1 (Table 3). To dissect the difference we did molecular docking of both PsA-SH and **12g** with HDAC1 and HDAC3

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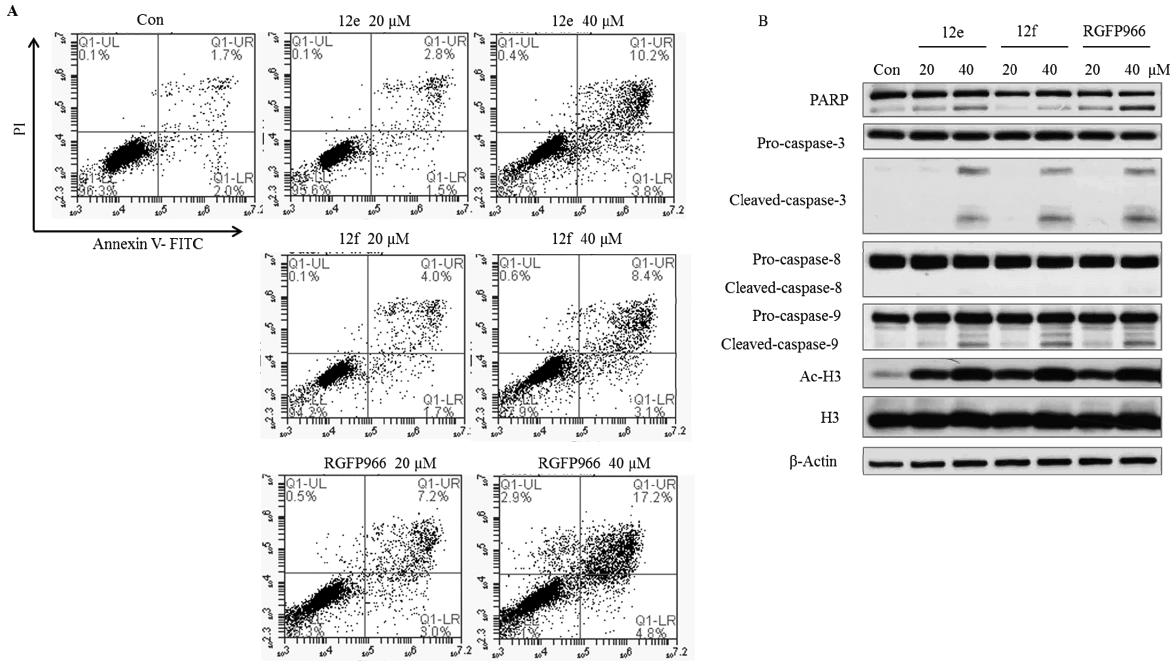

Figure 5. Apoptosis induction and protein regulation by **12e**, **12f**, and RGFP966 in HL-60 cells. Apoptotic cells were determined by FACS after staining with PI/Annexin-V (A), and the protein levels of Ac-H3, Ac-α-tubulin, PARP, caspase-3, -8, -9, and β-actin were determined by Western blotting (B) in HL-60 cells treated with **12e**, **12f**, and RGFP966 for 24 h.

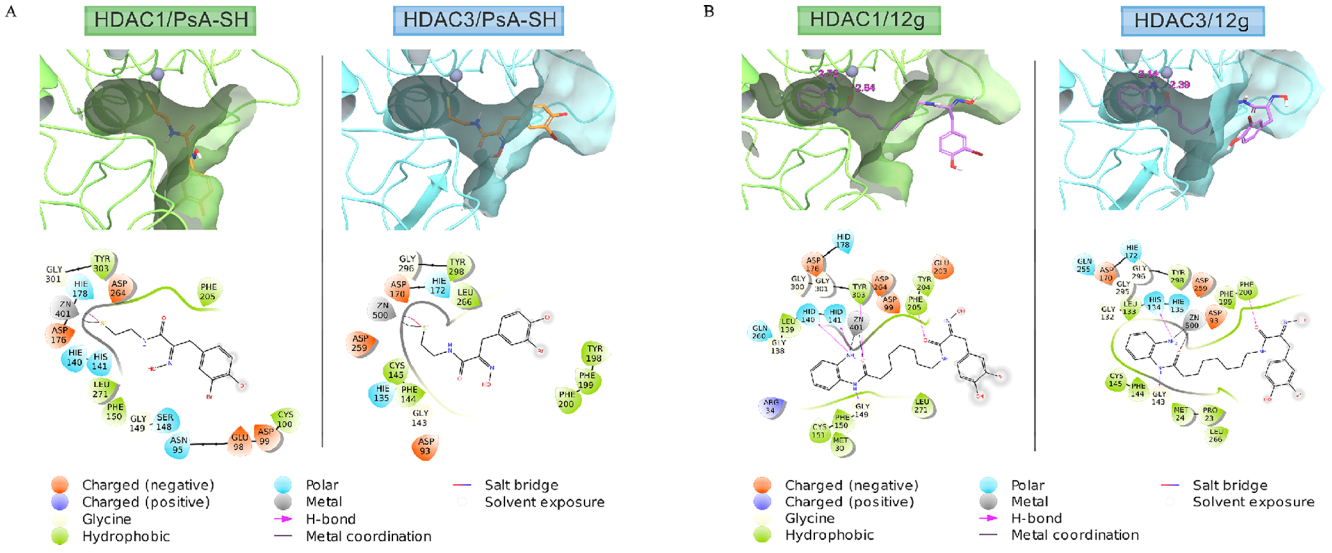

Figure 6. Putative binding patterns of PsA-SH (A) or **12g** (B) within HDAC1 (left panel, green) and HDAC3 (right panel, blue) pockets.

Table 4. Cell Growth Inhibition Profiles for PsA, PsA-SH, 10b, 12g, and RGFP966 in Four Solid Tumor Cell Lines

Compd.	MCF-7 IG_{50} [μM][a]	HCT116 IG_{50} [μM][a]	A549 IG_{50} [μM][a]	LL/2 IG_{50} [μM][a]
PsA	4.14 ± 0.06	2.3 ± 0.3	2.9 ± 0.7	2.64 ± 0.04
PsA-SH	17 ± 3	5.9 ± 0.6	10.7 ± 1.5	3.9 ± 0.7
10b	1.09 ± 0.02	0.90 ± 0.05	1.1 ± 0.4	5.0 ± 0.9
12g	17.9 ± 0.2	11.9 ± 1.7	26 ± 2	12.2 ± 1.0
RGFP966	30 ± 8	22 ± 4	28.3 ± 1.5	10.8 ± 0.2

[a]Values are means of at least three independent experiments.

protein. By aligning the crystal structures of HDAC1 (PDB code 5ICN)[35] and HDAC3 (PDB code 4A69)[36] we find that they share very similar binding pockets, with almost identical key amino acids within the binding pockets. PsA-SH exhibits very similar binding patterns within both HDAC1 and HDAC3 pockets, typically forming a coordinate bond with zinc ion (Figure 6A), suggesting that it could efficiently inhibit both of them. **12g** also exhibits very similar binding patterns within both HDAC1 and HDAC3 pockets; for example, the carbonyl and aniline group of **12g** forms a coordinate bond with the zinc ion, and another carbonyl group of **12g** is fixed through a hydrogen bond with Phe204 of HDAC1 and Phe200 of HDAC3 (Figure 6B). Comparison of the interaction distance between **12g** and the zinc ion reveals that **12g** demonstrates tighter contacts with the zinc of HDAC3 (2.14 Å for the aniline and 2.39 Å for the carbonyl group, respectively) than HDAC1 (2.72 Å for the aniline and 2.84 Å for the carbonyl

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Table 5. Antiproliferative Effects of PsA, PsA-SH, 10b, and SAHA on Lewis Lung Cancer LL/2 Cells in Mice

Group	Dose[mg/kg]	No. of mice	Body weight [g]		Tumor weight [g]	Inhibition rate [%]	*p value
			Initial	End			
Control	×12	5	18.4 ± 0.4	18.1 ± 0.2	0.98 ± 0.07		
PsA	25 mg/kg ×12	5	18.2 ± 0.5	18.4 ± 0.4	0.34 ± 0.09	66.04	<0.001
SAHA	25 mg/kg ×12	5	17.9 ± 0.5	18.1 ± 0.1	0.55 ± 0.07	45.77	<0.001
PsA-SH	25 mg/kg ×12	5	18.5 ± 0.8	18.3 ± 0.9	0.48 ± 0.11	55.99	<0.001
10b	25 mg/kg ×12	5	18.9 ± 0.4	18.4 ± 0.6	0.45 ± 0.04	54.68	<0.001

*p value compared with the control group.

Table 6. Antiproliferative Effects of 12g and PsA-SH on Lewis Lung Cancer LL/2 Cells in Mice

Group	Dose	No. of mice	Body weight [g]		Tumor weight [g]	Inhibitory [%]	*p value
			Initial	End			
Control	×12	5	18.6 ± 0.8	17.8 ± 0.7	1.2 ± 0.3		
12g	100 mg/kg ×12	5	18.6 ± 0.4	18.5 ± 0.3	0.8 ± 0.2	36.48	<0.001
PsA-SH	100 mg/kg ×12	5	18.6 ± 0.8	16.9 ± 0.4	0.5 ± 0.2	62.00	<0.001

*p value compared with the control group.

group, respectively), which may explain the relative selectivity of **12g** toward HDAC3 over HDAC1.

PsA, PsA-SH, **10b**, **12g**, and RGFP966 exhibited different abilities of inhibiting HL-60 cell growth (Tables 1−3). We extended their antiproliferative studies to several solid tumor cell lines including human breast cancer MCF-7 cells, colon cancer HCT116 cells, nonsmall cell lung cancer A549 cells, and marine Lewis lung cancer LL/2 cells (Table 4). PsA, PsA-SH, and **10b** are more effective than **12g** and RGFP966 to inhibit growth of those cell lines. **10b** is the most effective one among these compounds, and PsA is generally 2-fold more active than PsA-SH.

We used LL/2 cells to test the in vivo antitumor effects of PsA, PsA-SH, **10b**, and **12g** in mice. Since PsA, PsA-SH, and **10b** have similar antiproliferative effects in vitro, we gave the same doses of them to the mice for comparison. Mice inoculated with LL/2 cells were treated with each agent for 12 days at a dose of 25 mg/kg. The tumor growth inhibition rates of PsA, PsA-SH, and **10b** were 66.0%, 55.9%, and 54.7%, respectively. SAHA was used as a control with an inhibition rate of 45.8% (Table5). **12g** at 25 mg/kg did not exhibit antitumor effects. When the dose was increased to 100 mg/kg, **12g** only had an inhibition rate of 36.5%, less than that of PsA-SH at the same dose (Table 6). The body weights of mice did not decrease after treatment with each agent, suggesting that they are not toxic at the used doses. PsA, PsA-SH, and **10b** have similar antitumor effects in vitro and in vivo, and **12g** has decreased antitumor effects in vitro and in vivo. PsA, PsA-SH, **10b**, and SAHA have similar inhibition rates of tumor growth in vivo. Increased doses of PsA-SH could not significantly change the inhibition rate.

In summary, PsA-SH is the active molecule of PsA with abilities of inhibiting HDAC1/2/3 and inducing apoptosis. The −SH group plays an essential role in HDAC inhibition and apoptosis induction of PsA-SH. Depletion of the −SH group loses the abilities of HDAC inhibition and apoptosis induction. Replacement of the −SH group with the hydroxamic acid increases the ability of inhibiting HDAC6 which does not contribute to apoptosis induction. Replacement of the −SH group with the benzamide converts PsA-SH into a selective inhibitor of HDAC3 without apoptosis induction. Our data indicate that PsA-SH is a Class I HDAC inhibitor and a simple scaffold to design more selective HDAC inhibitors by modifying the ZBG.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00369.

Synthetic methods, characterization of compounds, biological assays for cell growth, enzyme activity, and Western blot, and high-solution mass spectra (PDF)

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Notes

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■ ABBREVIATIONS

Ac-H3, acetyl-Histone H3; COOH, carboxyl; DMSO, dimethyl sulfoxide; Et, ethyl; FACS, fluorescence-activated cell sorting; HDAC, histone deacetylase; IC_{50}, half maximal inhibitory concentration; IG_{50}, half the growth inhibition concentration; Me, methyl; MTT, methyl thiazolyltetrazolium bromide; OH, hydroxyl; PARP, poly-(ADP-ribose)-polymerase; PI, propidium iodide; PsA, psammaplin A; PMSF, phenylmethylsulfonylfluoride; PsA-SH, semipsammaplin A; SAHA, vorinostat; SDS, sodium dodecyl sulfate; -SH, sulfhydryl; SRM, surface recognition moiety; ZBG, zinc binding group

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