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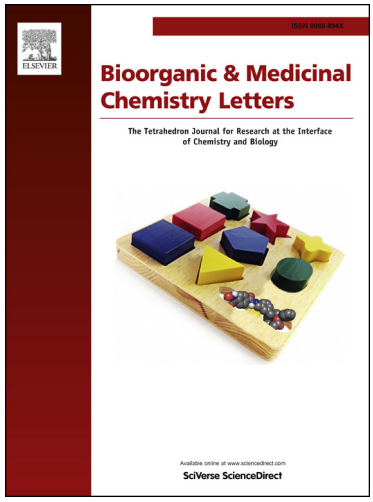

Synthesis, biological evaluation and molecular modeling studies ofPsammaplin A and its analogs as potent histone deacetylases inhibitors and cytotoxic agents

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Synthesis, biological evaluation and molecular modeling studies of Psammaplin A and its analogs as potent histone deacetylases inhibitors and cytotoxic agents

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Abstract:

In this study, a concise synthetic method of psammaplin A was achieved from 3-bromo-4-hydroxybenzaldahyde and hydantoin through a four–step synthesis via Knoevenagel condensation, hydrolysis, oximation and amidation in 37% overall yield. A collection of novel psammaplin A analogs focused on the variations of substituents at the benzene ring and modifications at the oxime moiety were synthesized. Among all the synthesized compounds, **5d** and **5e** showed better HDAC inhibition than psammaplin A and comparable cytotoxicity against four cancer cell lines (PC–3, MCF–7, A549 and HL–60). Molecular docking and dynamics simulation revealed that (i) hydrogen atom of the oxime group interacts with Asp99 of HDAC1 through a water bridged hydrogen bond and (ii) a hydroxyl group is optimal attached on the *para*-position of benzene, interacting with Glu203 at the entrance to the active site tunnel.

Keywords: Histone deacetylases inhibitors; Psammaplin A; Antiproliferation; Structure activity relationship; Molecular modeling

The acetylation and deacetylation processes of specific lysine residues on H3 or H4 histone tails play a key role in post–translational modification. Histone acetylases (HATs) and histone deacetylases (HDACs) are two families of enzymes that catalyze such processes. HDACs also participate in the regulation of non-histone proteins and are important in many biological processes such as transcriptional regulation, cell-cycle progression, cell survival and differentiation.[1] Eighteen HDAC isozymes in human have been identified and could be divided into two categories, the zinc–dependent enzymes (classes I, II, and IV) and the NAD^+–dependent enzymes (class III).[2,3]

Overexpression of classes I and II HDACs have been observed in many types of cancers and correlated with poor prognosis.[4-7] HDACs inhibition could cause proliferation inhibition, apoptosis, autophagy, differentiation, susceptibility to chemotherapy and migration inhibition of tumor cells.[8] Therefore, developing HDAC inhibitors (HDACIs) as anticancer drugs have attracted enormous attentions. A number of structurally diverse HDACIs have been identified, many of which are or derive from natural products.[9] Among them, Vorinostat , Romidepsin, Belinostat and Panobinostat have been approved for the treatment of cutaneous or peripheral T-cell lymphoma (CTCL or PTCL, **Figure 1**) and the general pharmacophore for HDACIs is composed of the Zn^{2+} binding group (ZBG), linker, and surface recognition motif (SRM).

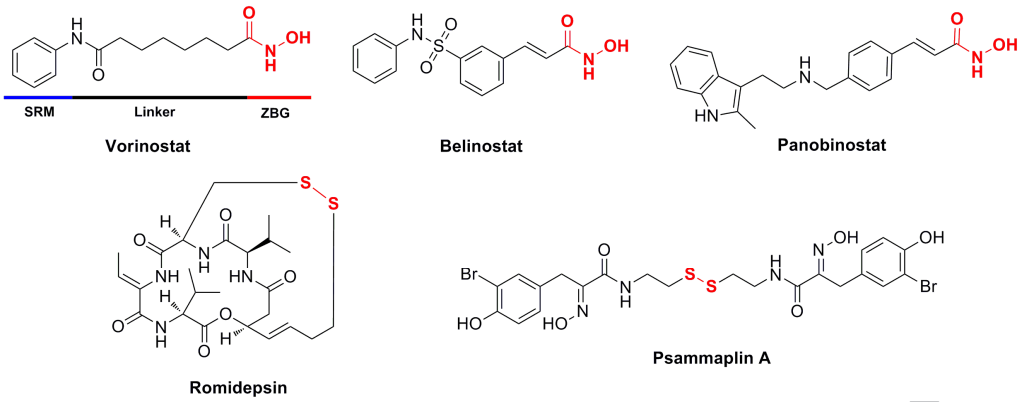
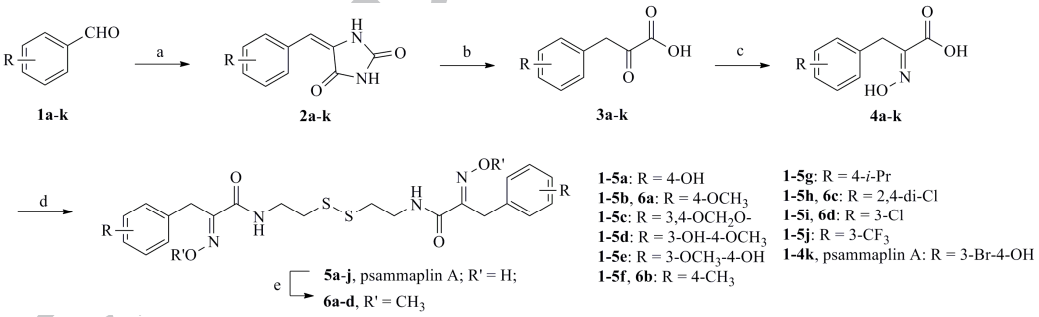


Figure 1. Structures of some documented HDACIs and its pharmacophoric characteristics.

Psammaplin A, first isolated from marine sponge in 1987, has been found to have a wide range of bioactivities, especially for antimicrobial and antitumor activity.[10] Recently, psammaplin A was revealed to be an epigenetic modulator targeting several epigenetic relevant targets, especially for HDACs.[11] As well as Romidepsin, psammaplin A is recognized as a nature prodrug and structure activity relationships have been enabled by several research groups.[11-15] The disulfide bond and the oxime moieties prove to be essential for its bioactivities, whereas structural variation at the SRM is tolerated. Based on these perspectives, a series of easy–to–prepare psammaplin A analogs bearing different substituents at the benzene ring (**5a**–**j**) were synthesized. On the other hand, the oxime moiety, reckoned as a metabolic issue fragment,[16-18] was replaced by methyl oxime (**6a**–**d**), pyrazole (**11a**–**c**) or isoxazole moiety (**11d**–**g**).

Restrained by the limited resource of natural derived psammaplin A, many research groups started its total synthesis. Some documented synthetic approaches to prepare psammaplin A were started from 4-hydroxyphenyl pyruvic acid or *L*-tyrosine and its derivatives, which produced psammaplin A in relatively poor yields. In addition, different

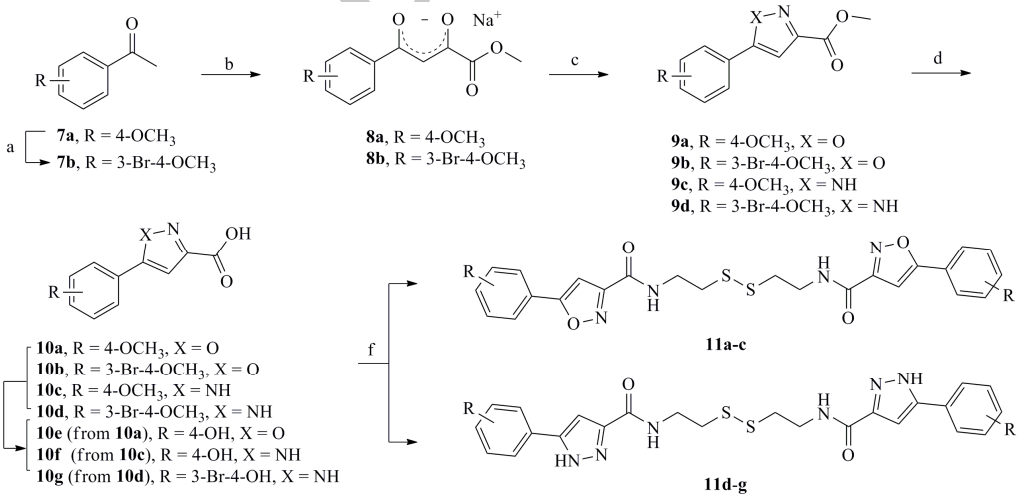
substituted tyrosine derivatives suffer limited commercial sources for use to explore the chemical space of SRM.[19,20] Previously, we have reported an alternative synthetic route of psammaplin A in good yield.[21] Herein, the synthetic approach was used in the preparation of its analogs with slight modification (**Scheme 1**). Briefly, benzalhydantoin **2k** was obtained from hydantoin and 3-bromo-4-hydroxybenzaldehyde **1k** through Knoevenagel condensation. After hydrolysis and oximation, the resulting α-oximic acid **4k** was coupled with cystamine dihydrochloride to yield psammaplin A through a very concise four–step synthesis in 37% overall yield. This synthetic method was favorable for preparing diverse analogs by taking appropriate benzaldehydes **1a–j** as crude material, yielding **5a–j** in good overall yields (32%–56%). O-methyloxime analogs **6a–d** were obtained through methylation of oximes (**5b**, **5f**, **5h** and **5i**) with methyl iodide in 82% to 91% yields. Detailed structures are given in **Table 1**.



Scheme 1. Synthesis of psammaplin A, **5a–j**, **6a–d**. Reagents and conditions: (a) hydantoin, ethanolamine, EtOH, H_2O, reflux, 5 h; (b) NaOH, H_2O, reflux, 12 h; (c) $NH_2OH{\cdot}HCl$, NaOH, $NaHCO_3$, H_2O, r.t., overnight; (d) cystamine dihydrochloride, EDCI, HOBt, THF, r.t., 24 h; (e) MeI, DMF, 65°C, 2 h.

The synthesis of phenylpyrazole and phenylisoxazole analogs **11a–g** is outlined in **Scheme 2**. 4-Methoxyacetophenone **7a** was brominated by NBS in the presence of 40%

sulfuric acid, yielding 3-bromo-4-methoxyacetophenone **7b** as a white powder. Next, after condensation with dimethyl oxalate, sodium ketoenolate ester **8a** and **8b** were produced in good yields. Treatment of the sodium ketoenolate ester with hydroxylamine hydrochloride in refluxing acetic acid yielded isoxazole ester **9a**–**b**, which were then hydrolyzed to produce 5-phenyl-isoxazole-3-carboxylic acids **10a**–**b**.[22] In addition, **8a**–**b** were readily converted into pyrazole ester **9c**–**d** by coupling with hydrazine hydrate, and then they were hydrolyzed into 5-phenyl-1*H*-pyrazole-3-carboxylic acids **10c**–**d**. Carboxylic acids **10a**–**d** treated with BBr_3 yielded phenols **10e**–**g**. It was unexpected that the demethylation form 5-(3-bromo-4-methoxyphenyl)isoxazole-3-carboxylic acid **10b** failed to produce desired phenol under the exact same protocol. The resulting carboxylic acids **10a**–**g** coupled with cystamine dihydrochloride afforded the required conjugates **11a**–**g** in 9%–48% overall yields. Detailed structures are given in **Table 2**.

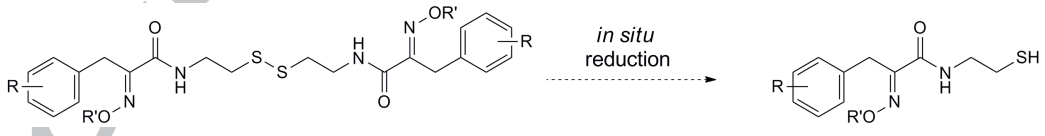


Scheme 2. Synthesis of **11a**–**g**. Reagents and conditions: (a) NBS, 40% H_2SO_4 in water, 60°C, 5 h; (b) dimethyl oxalate, NaOMe, Et_2O, r.t., 4 h; (c) $NH_2OH{\cdot}HCl$ or $NH_2NH_2{\cdot}H_2O$, AcOH,

reflux, 2 h; (d) LiOH, H_2O, dioxane, r.t., 16 h; (e) BBr_3, DCM, –20°C to r.t., 24 h; (f) cystamine dihydrochloride, EDCI, HOBt, THF, r.t., 24 h.

For the enzyme-based assay, strong HDAC inhibition (IC_{50}s at low submicromolar range) was observed in the benzene substituents modification molecules (**5a**–**j**). The replacement of 3-bromo-4-hydroxy group from psammaplin A decreased HDAC inhibition activity (**5a**–**c**, **5f**–**j**), while the 3-hydroxy-4-methoxy group **5d** and 3-methoxy-4-hydroxy group **5e** showed comparable, even better, enzyme inhibition than psammaplin A. Unfortunately, the oxime modification analogs **6a**–**d** and **11a**–**g** turned out to be less active than psammaplin A, indicating that the free oxime moiety is responsible for enzymatic inhibition, which is in line with the previous reports.[11-15,23,24] However, an interesting trend can be observed that phenylpyrazole analogs **11d**–**g** exhibited generally better HDAC inhibition than those phenylisoxazoles and O-methylated oximes. Although further exploration is required, it is apparent that the pyrazole N–H group could partially replace the free oxime group acting as a H-bond donor.

Table 1. Biological profiles of compounds **5a**–**j** and **6a**–**d**



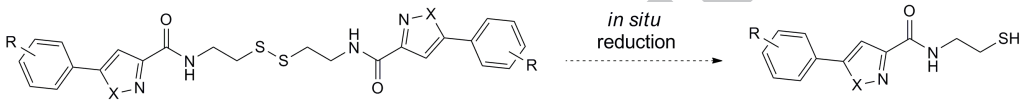
Compd.	R	R'	IC_{50} (μM)[a]	GI_{50} ± SD (μM)[b]			
			HDACs	PC–3	A549	MCF–7	HL–60
Psammaplin A	3-Br-4-OH	H	0.05	3.52 ± 0.11	4.50 ± 0.16	2.26 ± 0.26	0.29 ± 0.02
5a	4-OH	H	0.12	5.33 ± 0.18	6.54 ± 0.20	4.62 ± 0.11	0.62 ± 0.07
5b	4-OCH_3	H	0.12	5.50 ± 0.25	6.85 ± 0.48	2.68 ± 0.26	0.78 ± 0.13
5c	3,4-OCH_2O-	H	0.13	2.40 ± 0.49	4.78 ± 0.21	3.77 ± 0.65	0.67 ± 0.09
5d	3-OH-4-OCH_3	H	0.03	3.27 ± 0.25	4.74 ± 0.43	2.70 ± 0.30	0.23 ± 0.02
5e	3-OCH_3-4-OH	H	0.04	3.41 ± 0.15	4.84 ± 0.07	2.47 ± 1.07	0.28 ± 0.13

5f	4-CH_3	H	0.26	6.43 ± 0.19	8.35 ± 0.15	4.30 ± 0.69	0.74 ± 0.12
5g	4-*i*-Pr	H	0.21	2.67 ± 0.20	4.61 ± 0.18	3.91 ± 1.00	0.50 ± 0.03
5h	2,4-di-Cl	H	0.28	4.61 ± 0.31	6.79 ± 0.96	4.98 ± 0.50	0.87 ± 0.05
5i	3-Cl	H	0.21	5.19 ± 0.44	5.42 ± 0.20	3.51 ± 0.18	0.57 ± 0.16
5j	3-CF_3	H	0.27	6.78 ± 0.38	4.48 ± 0.51	3.48 ± 0.43	0.52 ± 0.05
6a	4-OCH_3	CH_3	3.15	26.80 ± 0.91	38.28 ± 0.60	16.39 ± 1.70	5.18 ± 0.70
6b	4-CH_3	CH_3	7.65	> 50	> 50	> 50	11.90 ± 0.51
6c	2,4-diCl	CH_3	8.59	> 50	47.09 ± 0.56	16.53 ± 1.03	8.64 ± 0.59
6d	3-Cl	CH_3	4.50	23.13 ± 0.08	32.02 ± 0.75	14.35 ± 0.58	6.55 ± 0.16

[a] Values are averages of at least two independent experiments, SD < 10%; Required pretreatment with TCEP to reduce the disulfide bond.

[b] Assays were performed at least three independent experiments, data are shown as mean ± SD.

Table 2. Biological profiles of compounds **11a–g**



Compd.	R	X	IC_{50} (μM)[a]	GI_{50} ± SD (μM)[b]	
			HDACs	PC–3	HL–60
11a	4-OCH_3	O	8.31	> 50	18.33 ± 0.22
11b	4-OH	O	3.14	31.67 ± 0.13	11.62 ± 0.54
11c	3-Br-4-OCH_3	O	4.65	38.96 ± 0.09	13.31 ± 0.42
11d	4-OCH_3	NH	2.77	26.55 ± 0.11	6.45 ± 0.52
11e	4-OH	NH	1.81	19.75 ± 0.07	4.11 ± 0.21
11f	3-Br-4-OCH_3	NH	1.86	16.90 ± 0.04	5.52 ± 0.67
11g	3-Br-4-OH	NH	0.68	17.08 ± 0.05	6.14 ± 0.55

[a] Values are averages of at least two independent experiments, SD < 10%; Required pretreatment with TCEP to reduce the disulfide bond.

[b] Assays were performed at least three independent experiments, data are shown as mean ± SD.

For the cytotoxicity assay, compounds **5d** and **5e** with the highest HDAC enzyme inhibition exhibited comparable antiproliferative activity with psammaplin A against all four cancer cells. Besides, **5c** and **5g** with medium potency showed greatest cytotoxicity against PC–3 cell line. IC_{50} values of **5c** and **5g** were 2.40 and 2.67 μM, respectively. The oxime modification compounds **6a–d** and **11a–g**, consistent with the enzyme activity, were partial or complete loss their cytotoxicity.

To further validate the target in cell-based model, compounds **5c–f** and **6b** were chosen to investigate the acetylating level of histone H3 and α-tubulin in human HL–60 leukemia cells. As shown in **Figure 2**, psammaplin A and **5c–f** greatly enhanced Ac-H3 at 2 μM instead of α-tubulin acetylation, suggesting a class I HDACs inhibition.[25] However, no Ac-H3 up-regulation was observed in **6b** under the assay conditions, which is in accord with the enzyme-based assay. Vorinostat as a pan-HDACI, the up-regulation of Ac–tubulin was already visible at 2 μM, while other compounds did not exhibited such an influence.

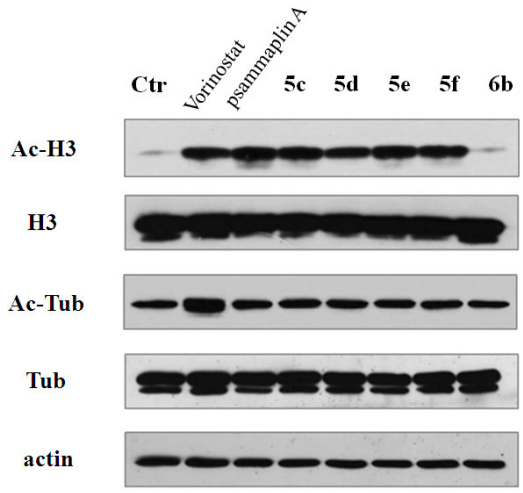


Figure 2. Effects on histone-H3, acetylated histone-H3, α-tubulin and acetylated α-tubulin of Vorinostat, psammaplin A, **5c**, **5d**, **5e**, **5f** and **6b** in HL–60 cells. Cells were treated with compounds (2 μM) for 18 h.

In light of the above-mentioned bioactivity results, **5d** and **5e** were found to exhibit both good HDAC inhibition activity and cytotoxicity. Thus, a docking analysis was carried out to compare the potential binding modes of psammaplin A and its analogs with HDAC1 (PDB ID: 4BKX).[26] As shown in **Figure 3A**, the reduced psammaplin A binds to HDAC1 and forms key interactions with the protein in several areas. In detail, the thiol group chelates the Zn^{2+}

ion, the oxime group forms a H-bond with Asp99 bridged by a water. As to the surface recognition motif, the 3-bromo-4-hydroxy phenyl group forms few hydrophobic contacts to His178, Tyr204 and Phe205 and the hydroxyl group forms additional hydrogen bonds with Glu203 bridged by a water. The non-covalent interactions between the inhibitor and target protein are quite stable through 2.5 ns dynamic simulation (supporting information). **5a–j** share a similar binding mode as psammaplin A, as exemplified by **5e** shown in **Figure 3B**. On the contrast, O-methylated oximes **6a–d** as well as oxime cyclized analogs **11a–g** fail to interact with the key amino acid residue Asp99, leading to binding energy reduction.

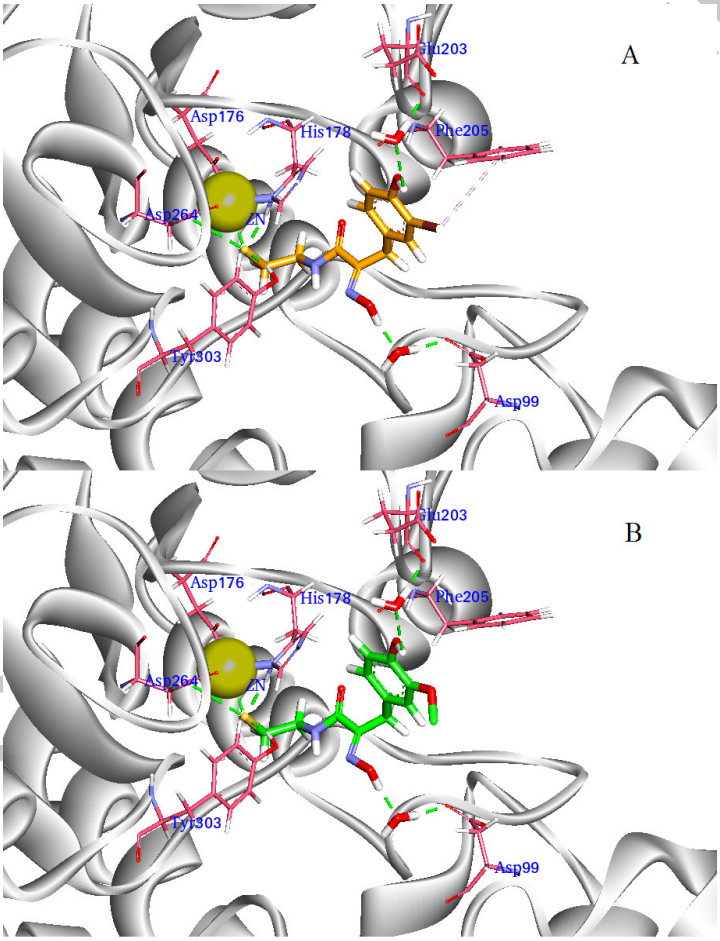


Figure 3. Stereoview of the simulated docking pose of reduced psammaplin A (A) and **5e** (B) to HDAC1 (4BKX) are represented in orange and green, respectively. Important parts of the

enzyme for interaction are shown in magenta sticks. The zinc ion is shown as a light yellow sphere.

In conclusion, we have developed a concise synthetic method of psammaplin A through a four–step (37% overall yield) synthesis from 3-bromo-4-hydroxybenzaldahyde and hydantoin via Knoevenagel condensation, hydrolysis, oximation and amidation. A collection of novel psammaplin A derivatives focused on the variations of substituents at the benzene ring and modifications at the oxime moiety were synthesized through this synthetic approach. Structure-activity relationship study supported that the free oxime group and appropriate benzene substituents were necessary for high HDACs inhibition and cytotoxicity. Among all the synthesized compounds, **5d** and **5e** showed better HDAC inhibition than psammaplin A and comparable cytotoxicity against several cancer cell lines. Molecular docking and dynamics simulation indicated that (i) hydrogen atom of the oxime group interacts with Asp99 of HDAC1 through a water bridged hydrogen bond and (ii) a hydroxyl group is optimal attached on the *para*-position of benzene, thus interacting to Glu203 at the entrance to the active site tunnel.

Acknowledgments

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Graphical abstract