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Evaluation of antiangiogenic activity of azumamides by the in vitro vascular organization model using mouse induced pluripotent stem (iPS) cells

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Abstract—Evaluation of antiangiogenic activity of marine sponge derived azumamides by the in vitro vascular organization model using mouse induced pluripotent stem (iPS) cells was carried out. Azumamide E (**5**) strongly inhibited in vitro angiogenesis from iPS cells at 1.9 μM while azumamide A (**1**) showed only weak inhibition at 19 μM. These results were well correlated with HDAC inhibitory activity of these compounds, revealing the prospect of azumamides as the probe molecules useful for stem cell chemical biology.

The establishment of mouse induced pluripotent stem (iPS) cells[1] followed by the success in reprogramming of differentiated human somatic cells into a pluripotent state[2,3] gave a significant impact on the life sciences not only because of the possibility of regeneration therapy without rejection associated with traditional transplantation but also because of the prospect for application to the assay systems evaluating efficacy and toxicity of drugs against the individual SNP. Therefore, it seems to be an urgent issue to verify the biological activity of the probe molecules found by the assay using ES cells in the iPS systems for further development of stem cell biology by means of chemical biological strategy.

Azumamides A–E (**1**–**5**) are the histone deacetylase (HDAC) inhibitors isolated from the marine sponge *Mycale izuensis*.[4,5] Consistent with the former report that HDACs can be the promising target of antiangiogenic therapy,[6,7] azumamide A (**1**) was also found to inhibit vascularization in the in vitro vascular organization model using mouse ES cells at 19 μM.[8]

Since the following studies[9,10] revealed that the HDAC inhibitory activity of synthetic **1** was not as potent as that of originally reported while synthetic **5** retained the equivalent potency, we re-evaluated inhibitory activities of **1**–**5** against HDAC1, HDAC4, and HDAC6, as well as p21 promotion activities. Then, the antiangiogenic activities of azumamides A (**1**) and E (**5**) by the in vitro vascular organization model using mouse induced pluripotent stem (iPS) cells were tested. As a result, we found a dose-dependent antiangiogenic activity in the iPS cell system of **5**, while **1** showed only a moderate effect.

These results were well correlated with their HDAC inhibitory activities, and therefore, azumamide E (**5**) is presumed to be the promising probe molecule for the chemical biology of angiogenesis and stem cell differentiation.

Azumamides A–E were assayed for HDAC inhibitory activity using HDAC1, HDAC4, and HDAC6 enzymes prepared from 293T cells.[11] Azumamides A (**1**) and D (**4**) showed only a very weak inhibitory activity against

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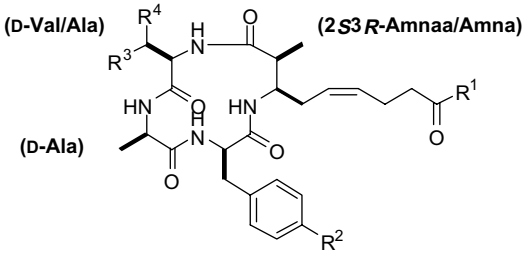
these subtypes at 50 μM, while B (**2**), C (**3**), and E (**5**) showed inhibitory activities against HDAC1 and HDAC4 with IC_{50} values ranging from 1.17 to 3.66 μM. None of **1**–**5** showed remarkable activity against HDAC6. Promotion of p21 expression was also evaluated[12] and only azumamide E (**5**) showed a moderate activity with EC_{1000} value of 17.0 μM (Table 1).



		R^1	R^2	R^3 = R^4
azumamide	A (1)	NH_2	H	Me
	B(2)	NH_2	OH	Me
	C(3)	OH	OH	Me
	D(4)	NH_2	H	H
	E(5)	OH	H	Me

These results suggested that azumamide E (**5**) is the most active among **1**–**5**, therefore, **1** and **5** were tested for their antiangiogenic activity in the in vitro vascular organization model using mouse iPS cells.[13]

Evaluation of antiangiogenic activity was carried out basically following the procedure using mouse ES cells,[4,8] except that mouse iPS cells derived from mouse skin fibroblasts were used instead of mouse ES cells.

In this model, azumamide A (**1**) did not show or showed only a weak inhibition even at 19 μM (Fig. 1c and g), which is inconsistent to the former result but consistent with the HDAC inhibitory activity (IC_{50} > 50 μM) obtained in this assay.

On the other hand, azumamide E (**5**) showed dose-dependent inhibition of angiogenesis in this model at as low as 0.19 μM (Fig. 2). The observed antiangiogenic activity of **5** was again well consistent with the inhibitory activity against HDAC1 and HDAC4.

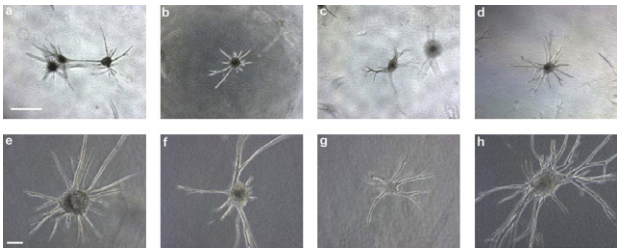


Figure 1. Effects of azumamide A (**1**) on in vitro vascular organization of mouse iPS cells. (a–d) ×4, bar = 500 μm. (e–h) ×10, bar = 100 μm. (a and e) 0.19 μM of **1**. (b and f) 1.9 μM of **1**. (c and g) 19 μM of **1**. (d and h) 0 μM of **1**.

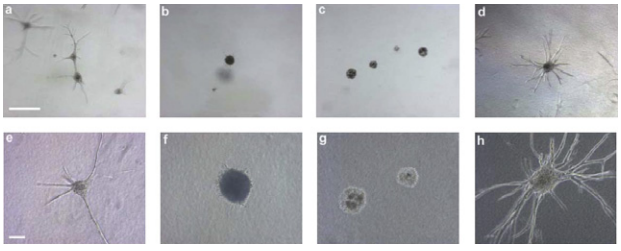


Figure 2. Effects of azumamide E (**5**) on in vitro vascular organization of mouse iPS cells. (a–d) ×4, bar = 500 μm. (e–h) ×10, bar = 100 μm. (a and e) 0.19 μM of **5**. (b and f) 1.9 μM of **5**. (c and g) 19 μM of **5**. (d and h) 0 μM of **5**.

In the present study, we have tested inhibitory activity of azumamides A–E (**1**–**5**) against HDAC1, HDAC4, and HDAC6, as well as p21 promotion activity. In the HDAC inhibitory assay system used in this study, azumamides showed weaker activities than those obtained in the previous report.[4] Inhibitory activities obtained for the synthetic azumamides by Ganesan[10] also showed a weaker activity of **1**. From the results of De Riccardis[9] and Ganesan,[10] it seems that assay conditions may largely affect the HDAC inhibitory activities. However, the results of HDAC inhibition assay for azumamides A and E in the present study were well correlated with the antiangiogenic activity in the iPS cells system.

It will be a valid strategy for the stem cell chemical biology, to compare gene expression or epigenetic states during the cell differentiation processes under the different culture conditions prepared by the administration of positive or negative probe molecules, respectively. Azumamides A (**1**) and E (**5**) can be such a probe molecule

Table 1. Biological activity of azumamides A–E (**1**–**5**)

Compound	HDAC1[a]	HDAC4[a]	HDAC6[a]	p21 promotion[b]
1	>50 μM	>50 μM	>50 μM	>25 μM
2	1.83 μM ± 0.11	3.66 μM ± 1.34	>50 μM	>25 μM
3	1.17 μM ± 0.16	3.16 μM ± 0.21	>50 μM	>25 μM
4	>50 μM	>50 μM	>50 μM	>25 μM
5	1.22 μM ± 0.13	2.28 μM ± 0.16	>50 μM	17.0 μM ± 3.91
TSA[c]	0.0366 μM	0.0629 μM	0.0833 μM	0.0115 μM

[a] IC_{50} values (±standard deviation).
[b] EC_{1000} values.
[c] Trichostatin A.

set ideal for the chemical biological study of stem cell differentiation and angiogenesis using iPS cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.053.

References and notes

1. Takahashi, K.; Yamanaka, S. *Cell* **2006**, *126*, 663.
2. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. *Cell* **2008**, *131*, 1.
3. Yu, J.; Vodyanik, M. A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J. L.; Tian, S.; Nie, J.; Jonsdottir, G. A.; Routti, V.; Stewart, R.; Slukvin, I. I.; Thomson, J. A. *Science* **2007**, *318*, 1917.
4. Nakao, Y.; Yoshida, S.; Matsunaga, S.; Shindoh, N.; Terada, Y.; Nagai, K.; Yamashita, J. K.; Ganesan, A.; van Soest, R. W. M.; Fusetani, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 7553.
5. Izzo, I.; Maulucci, N.; Bifulco, G.; De Riccardis, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 7557.
6. Qian, D. Z.; Kato, Y.; Shabbeer, S.; Wei, Y.; Verheul, H. M. W.; Salumbides, B.; Sanni, T.; Atadja, P.; Pili1, R. *Clin. Cancer Res.* **2006**, *12*, 634.
7. Chang, S.; Young, B. D.; Li, S.; Qi, X.; Richardson, J. A.; Olson1, E. N. *Cell* **2006**, *126*, 321.
8. Yamashita, J.; Itoh, H.; Hirashima, M.; Ogawa, M.; Nishikawa, S.; Yurugi, T.; Naito, M.; Nakao, K.; Nishikawa, S.-I. *Nature* **2000**, *408*, 92.
9. Maulucci, N.; Chini, M. G.; Di Micco, S.; Izzo, I.; Cafaro, E.; Russo, A.; Gallinari, P.; Paolini, C.; Nardi, M. C.; Casapullo, A.; Riccio, R.; Bifulco, G.; De Riccardis, F. *J. Am. Chem. Soc.* **2007**, *129*, 3007.
10. Wen, S.; Carey, K. L.; Nakao, Y.; Fusetani, N.; Packham, G.; Ganesan, A. *Org. Lett.* **2007**, *9*, 1105.
11. Shivashimpi, G. M.; Amagai, S.; Kato, T.; Nishino, N.; Maeda, S.; Nishino, T. G.; Yoshida, M. *Bioorg. Med. Chem.* **2007**, *15*, 7830.
12. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916.
13. Narazaki, G.; Teranishi, M.; Okita, K.; Yamanaka, S.; Yamashita, J. K. Presented at the 7th Congress of Japan Society for Regenerative Medicine, Nagoya, Japan, March 2008, paper o-21-1.