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Synthesis and antitumor activity of cyclodepsipeptide zygosporamide and its analogues

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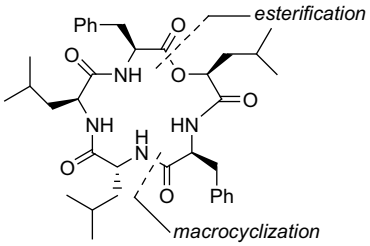
ABSTRACT

The synthesis and structure–activity relationships of zygosporamide, a known potent and selective cytotoxic natural product against SF-268 and RXF 393 cell lines, are described. The potencies of the synthetic zygosporamide are similar to those reported for the natural product toward all cancer cell lines examined with the exception of SF-268, the underlying cause of which remains to be elucidated.

Zygosporamide (**1**) is a cyclic depsipeptide that was isolated from the seawater-based fermentation broth of a marine-derived fungus identified as *Zygosporium masonii* (Fig. 1).[1] It is composed of four hydrophobic amino acids (D-Leu, L-Leu, and two L-Phe) and one hydrophobic hydroxy acid ((*S*)-2-hydroxy-4-methylpentanoicacid; *O*-Leu). In the National Cancer Institute 60 cancer cell lines screen, this compound displayed highly selective cytotoxicity against CNS (central nervous system) cancer cell line SF-268 and renal cancer cell line RXF 393, with GI_{50} values of 6.5 nM and less than 5.0 nM, respectively. These values are at least 1000× lower than most of the 54 (out of 60) cancer cell lines tested. The unusual cell type selectivity displayed by this structurally simple compound prompted us to initiate a synthetic and SAR program toward zygosporamide. It is our long-term goal to explore the mode of action by this natural product, particularly its selectivity toward different cancer cell lines.[2]

Since zygosporamide contains a D-leucine that is connected with L-phenylalanine, we decided to carry out the macrocyclization at this site (Fig. 1).[3] The cyclization precursor could be assembled via ester bond formation. Based on this analysis, we started the total synthesis by preparing tripeptide **4b** as depicted in Scheme 1. Condensation of *N*-Cbz-D-leucine with L-leucine methyl ester under the action of EDC, HOBt, and DIPEA provided dipeptide **3** in 92% yield. After hydrolysis of **3** with aqueous LiOH in THF and MeOH, the resultant acid was condensed with L-phenylalanine methyl ester to afford amide **4a**, which was then hydrolyzed with LiOH to give the tripeptide **4b**.



zygosporamide (**1**)

Figure 1. Structure and bond-disconnections of zygosporamide.

In a parallel procedure, acid **5** was condensed with the *p*-toluenesulfonic acid salt of L-Phe-OBn to produce amide **6** in 90% yield. Esterification of **4a** with **6** under Yamaguchi conditions[4] gave rise to ester **7**. After hydrogenolysis of **7**, the macrocyclization precursor was obtained. As we anticipated, the HATU-mediated macrolactamization of this amino acid proceeded smoothly in a mixture of DMF and methylene chloride to furnish the cyclic depsipeptide **1**[5] in 50% yield. Its analytical data were identical to those previously reported.[1]

Alanine-scan technique is a classical tool to study the structure–activity relationship of proteins as well as smaller polypeptides.[6] Because the chirality of Ala is identical to that of the corresponding residue in parent peptides, substitution of other amino acids with Ala is not expected to cause significant conformational changes. Following the above procedure, we synthesized four Ala-substituted analogues of zygosporamide (**8**–**11**, Fig. 2) by

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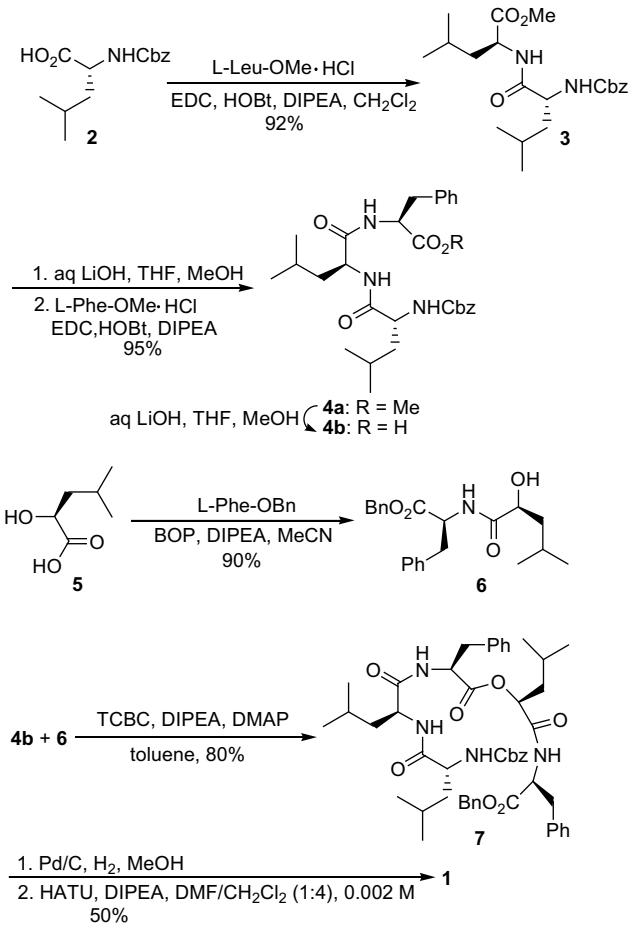

Scheme 1.

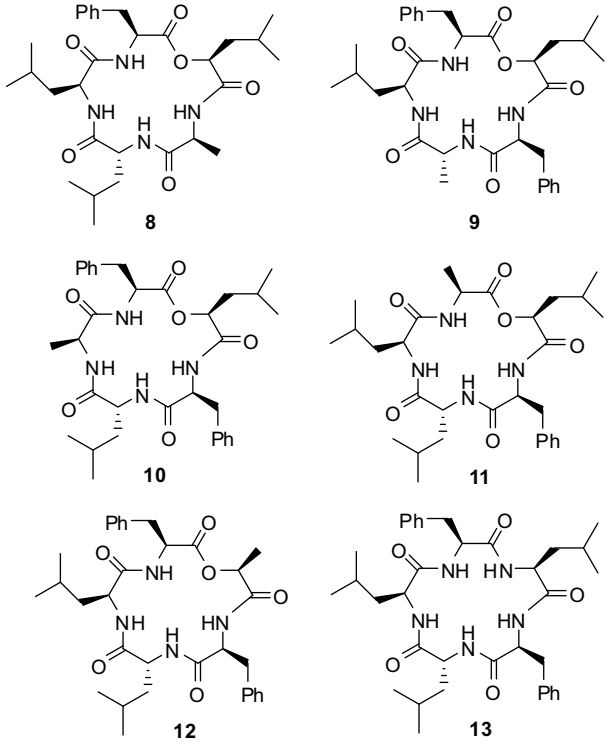

Figure 2. Structures of synthetic analogues of zygosporamide.

replacing each amino acid residue with Ala, in order to determine which residue(s) plays an important role in the interaction with the putative cellular target. In addition, a L-lactic acid substituted cyclodepsipeptide **12** and a cyclic pentapeptide **13** were elaborated to see if the amide unit is important for antitumor activity. Noteworthy is that the similar modification to cyclic depsipeptide, sansalvamide A, has resulted in some new potent antitumor agents.[7]

Before testing the synthetic zygosporamide and its analogues in cancer cell lines, we determined the IC_{50} values of paclitaxel as a reference compound in our cell proliferation assay. As shown in Table 1, the cytotoxicity of paclitaxel in five tested cancer cell lines (SF-268, SF-295, A549, MDA-MB-231, and HCT-116) is comparable to that previously reported, which validated our assay conditions.

Using the same assay, we next determined the cytotoxicity of the synthetic zygosporamide and its analogues. The results for inhibition of the growth of two CNS cancer cell lines (SF-268 and SF-295), a lung cancer cell line (A549), a breast cancer cell line (MDA-MB-231), and a colon cancer cell line (HCT-116) are summarized in Table 2. The IC_{50} values for the synthetic zygosporamide (**1**) against most cell lines including SF-295, A549, MDA-MB-231, and HCT-116 are similar to those previously reported for the natural product.[1] A notable exception, however, is the IC_{50} value of the synthetic zygosporamide (**1**) against SF-268 (4.6 ± 0.7 μM), which is about 700-fold higher than that previously reported. The underlying cause of the discrepancy in potencies against SF-268 cell line between our synthetic sample and the natural product remains unknown.[8] It is possible that the SF-268 cells we received from ATCC have undergone further changes upon passage in cell culture, losing the unique genetic or epigenetic characteristics that conferred its unusually high sensitivity to zygosporamide in the previous study.

Taking advantage of our new synthetic route to zygosporamide, we made several analogues for a preliminary structure–activity relationship study. Most synthetic analogues (**8–13**) showed lower cytotoxicity toward SF-268, SF-295, and A549 in comparison with the synthetic zygosporamide (**1**). However, analogue **11** exhibited slightly higher cytotoxicity and analogues **8–9** showed similar cytotoxicity toward MDA-MB-231. In addition, the cytotoxicity of analogues **9** and **11–13** was considerably higher than that of synthetic zygosporamide toward HCT-116. Thus, Ala[2]-substituted analogues**10** did not show significant cytotoxicity at the highest concentration tested (50 μM), while Ala[5]-substitued analogues **8** only had limited cytotoxicity. This indicates that L-Leu[2] and L-Phe[5] side-chain functional groups are indispensable for the cellular activity. The potencies of the L-lactic acid–substituted cyclodepsipeptide **12** and the cyclic pentapeptide **13** were more than 8-fold and more than 4-fold lower than the compound **1** (except for HCT-116), respectively, which suggests that O-Leu side-chain and the ester linkage are preferred at this position at least for cytotoxicity toward certain cancer cell lines. In addition, substituted analogues **9** and **11** generally maintained

Table 1
In vitro cytotoxicity data for paclitaxel toward SF-268, SF-295, A549, MDA-MB-231, and HCT-116 cell lines

Cell line	IC_{50} (nM) Paclitaxel	IC_{50} (nM)* NSC125973**
SF-268	15.3 ± 2.7	25
SF-295	290 ± 220	63
A549	23.1 ± 1.8	25
MDA-MB-231	40.5 ± 7.6	50
HCT-116	13.6 ± 2.4	6.3

* IC_{50} values in this column are from the data base of DTP NCI/NIH (http://www.dtp.nci.nih.gov/).
** In DTP data base, compound NSC125973 is paclitaxel.

Table 2
In vitro cytotoxicity data for synthetic zygosporamide and its analogues toward SF-268, SF-295, A549, MDA-MB-231 and HCT-116 cell lines

compound	IC_{50} (μM) SF-268	IC_{50} (μM) SF-295	IC_{50} (μM) A549	IC_{50} (μM) MDA-MB-231	IC_{50} (μM) HCT-116
1	4.6 ± 0.7	4.2 ± 1.5	2.5 ± 0.5	5.7 ± 2.1	~22*
8	~35*	19.7 ± 8.8	~31*	7.5 ± 6.3	>50
9	10.4 ± 1.5	8.7 ± 0.9	>50	5.0 ± 1.8	2.1 ± 0.4
10	>50	>50	>50	>50	>50
11	8.8 ± 1.5	~6*	>50	2.8 ± 2.1	2.7 ± 0.6
12	~31*	~32*	>50	>50	7.8 ± 6.0
13	20.5 ± 2.0	14.4	21.0 ± 5.9	>50	1.9 ± 1.3
Zygosporamide**	0.0065	15	7.4	8.5	11.5

* Those dose–response curves are not sigmoid curves.
** Values from Ref. 1.

moderate activities. These data suggest that the hydrophobic group of D-Leu[1] and the phenyl group of L-Phe[3] are not absolutely required for activity. Hence, the preliminary analysis of structure–activity relationships reveals that the hydrophobic group of D-Leu[1] may be a good target for further rational design, and the phenyl group of L-Phe[3] or L-Phe[5] also could be further optimized to generate new compounds with higher potency against proliferation of certain cancer cells.

In summary, we have achieved the first total synthesis of zygosporamide and found that the synthetic compound possessed similar cytotoxicity toward SF-295, A549 and MDA-MB-231 and HCT-116, but a significantly lower potency against SF-268 in comparison with that reported for natural zygosporamide. The preliminary SAR studies reported here should be helpful for further development of more potent antitumor compounds. Investigation in this direction is being actively pursued in this group and will be reported elsewhere in due course.

Acknowledgments

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