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Marine spongian sesquiterpene phenols, dictyoceratin-C and smenospondiol, display hypoxia-selective growth inhibition against cancer cells

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Abstract

In the course of our search for hypoxia-selective growth inhibitors against cancer cells, a sesquiterpene phenol, dictyoceratin-C (**1**), was isolated from the Indonesian marine sponge of *Dactylospongia elegans* under the guidance of the constructed bioassay. Dictyoceratin-C (**1**) inhibited proliferation of human prostate cancer DU145 cells selectively under hypoxic condition in a dose-dependent manner at the concentrations ranging from 1.0 to 10 μM. The subsequent structure-activity relationship study using nine sesquiterpene phenol/quinones (**2**-**10**), which were isolated from marine sponge, was executed. We found that smenospondiol (**2**) also exhibited the similar hypoxia-selective growth inhibitory activity against DU145 cells, and the *para*-hydroxybenzoyl ester moiety would be important for hypoxia-selective growth inhibitory activity of **1**. In addition, the mechanistic analysis of dictyoceratin-C (**1**) revealed that the 10 μM of **1** inhibited accumulation of Hypoxia-Inducible Factor-1α under hypoxic condition.

Key words: dictyoceratin-C; smenospondiol; HIF-1α; hypoxia; cancer; *Dactylospongia elegans*

Text

It is now generally accepted that cancer cells, which have adapted to the hypoxic environment in tumor tissues, aggravate pathology of cancer by promoting tumor growth, angiogenesis, metastasis, and drug resistance.[1] In addition, hypoxic environment in a tumor is unlike that in normal tissues. Therefore, compounds that inhibit growth of tumor cells selectively under the hypoxic environment are expected to represent promising new leads for anti-cancer drugs. Recently, we established a new screening system searching for hypoxia-selective growth inhibitors to explore new leads for anti-cancer drugs.[2] Using this screening system, we isolated a furanosesterterpene, furospinosulin-1,[3] from the Indonesian marine sponge of *Dactylospongia elegans*. Furospinosulin-1 showed selective anti-proliferative activity against human prostate cancer DU145 cells under the hypoxic condition, and it also exhibited potent anti-tumor activity under oral administration.[4] The mechanistic analysis revealed that furospinosulin-1 suppressed transcription of the *insulin-like growth factor-2* (*IGF-2*) gene, which is selectively induced under the hypoxic condition, through preventing the binding of nuclear proteins to the Sp1 consensus sequence in the promoter region of

IGF-2 gene.[4]

In our continuing search for hypoxia-selective growth inhibitors against cancer cells from the marine sponge of *Dactylospongia elegans*, a sesquiterpene phenol, dictyoceratin-C (**1**),[5] was isolated under the guidance of the constructed bioassay (Fig. 1).[6] When human prostate cancer DU145 cells were incubated for 24 h in the presence of dictyoceratin-C (**1**) under normoxic or hypoxic conditions, dictyoceratin-C (**1**) inhibited proliferation of DU145 cells selectively under the hypoxic condition in a dose-dependent manner at the concentrations ranging from 1.0 to 10 μM. Maximal growth inhibition of 43 % was achieved with 10 μM of dictyoceratin-C (**1**) under the hypoxic condition. Although the higher dose (30 μM) of **1** showed significant anti-proliferative activity against DU145 cells, selectivity between normoxic and hypoxic conditions became low (Fig. 2).

We have previously isolated eight sesquiterpene phenol/quinones including dictyoceratin-C (**1**) from the same marine sponge of *Dactylospongia elegans* in the study searching for differentiation-inducing substances in human chronic myelogenous leukemia K562 cells.[7] The structure-activity relationship study using these compounds

indicated that the amino-quinone moiety is indispensable for their differentiation-inducing activity.[7] In addition, we also have reported that two sesquiterpene quinones (dysideamine (**9**) and bolinaquinone (**10**)) having unique rearranged drimane skeleton, which were isolated from an Indonesian marine sponge of *Dysidea* sp., exhibited the neuroprotective effect against iodoacetic acid-induced HT22 cell death at 10 μM concentration.[8] In an effort to clarify structure-activity relationship for the hypoxia-selective growth inhibitiory activity of dictyoceratin-C (**1**), we investigated anti-proliferative activity of these nine sesquiterpene phenol/quinones (smenospondiol (**2**), smenospongine (**3**), smenospongorine (**4**), ilimaquinone (**5**), 5-*epi*-smenospongine (**6**), 5-*epi*-smenospongorine (**7**), 5-*epi*-smenospongidine (**8**), dysideamine (**9**) and bolinaquinone (**10**))[7, 8] against DU145 cells under both normoxic and hypoxic conditions (Fig. 1 and 2). Among them, only smenospondiol (**2**) showed similar selective anti-proliferative activity against DU145 cells under hypoxic condition at the concentrations ranging from 1.0 to 10 μM. While, the other compounds **3**-**10** having hydroxyquinone moiety did not show hypoxia-selective growth inhibition. These findings suggested that the *para*-hydroxybenzoyl ester moiety would be important for

hypoxia-selective growth inhibitiory activity of **1** against DU145 cells.

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcription factor, which comprises an oxygen-regulated α-subunit and a constitutively expressed β-subunit. Under hypoxic conditions, O_2-dependent hydroxylation of HIF-1α subunit, which leads a degradation by the proteasome, is inhibited, and the resulting accumulated HIF-1α dimerized with HIF-1β and activates transcription of target genes. HIF-1 activation promotes oncogenesis and/or cancer progression.[9, 10] Furthermore, inhibition of HIF-1 activity leads to decrease vascular endothelial cell growth factor (VEGF) expression. Therefore, HIF-1 has been paid much attention as a drug target for cancer chemotherapy. In order to clarify action-mechanism of the hypoxia-selective growth inhibitory activity against DU145 cells by dictyoceratin-C (**1**), we performed western blotting analysis to examine accumulation of HIF-1α and production of VEGF, which is regulated by HIF-1.[11] As shown in Figure 3, DU145 cells increased accumulation of HIF-1α and production of VEGF under the hypoxic condition. The both 10 and 30 μM concentrations of dictyoceratin-C (**1**) inhibited hypoxia-induced accumulation of HIF-1α. While, the production of VEGF was slightly inhibited by 10 μM of

dictyoceratin-C (**1**), and the treatment with 30 μM of compound **1** reduced production of VEGF to the level of the normoxic condition. [12] On the other hand, dysideamine (**9**) did not inhibit accumulation of HIF-1α and production of VEGF under the hypoxic condition. These observations suggested that the hypoxia-selective growth inhibition against DU145 cells by dictyoceratin-C (**1**) would be due to its ability to prevent accumulation of HIF-1α. The anti-tumor activity and the target protein of dictyoceratin-C (**1**) and smenospondiol (**2**) are currently being studied.

Acknowledgements

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References and notes

1. Dewhirst, M. W.; Cao, Y.; Moeller, B. *Nat. Rev. Cancer* **2008**, *8*, 425.
2. Method for bioassay: Human prostate cancer DU145 cells in the RPMI 1640 medium supplemented with heat-inactivated 10% fetal bovine serum (FBS) were plated onto 96-well plates (1×10^4 cells/well/200 μL) for 4 h in a humidified atmosphere of 5% CO_2 at 37 ºC (normoxic condition). Then, the plates were incubated for 12 h in the normoxic condition or in the atmosphere of 94 % nitrogen, 5% CO_2, and 1% O_2 (hypoxic condition) inducing hypoxia-related genes such as HIF-1α. After the 12 h incubation, the testing compounds were added, and the plates were then incubated for an additional 24 h under normoxic or hypoxic conditions. The cell proliferation was detected by the MTT method. The growth inhibition rate was calculated as the percentage of parallel negative controls. Data are shown as mean ± SD and represented as the mean of 3 separate experiments performed in triplicate.
3. Cimino, G.; de Stefano, S.; Minale, L. *Tetrahedron* **1972**, *28*, 1315.
4. Arai, M.; Kawachi, T.; Setiawan, A.; Kobayashi, M. *ChemMedChem* **2010**, *5*, 1919.
5. Kushlan, D. M.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. *Tetrahedron* **1989**, *45*, 3307.
6. The MeOH extract of the dried marine sponge (370 g) of *Dactylospongia elegans* 01A10, which was collected in 2001 at Indonesia, was partitioned into a water-EtOAc mixture. The EtOAc soluble portion (54 g) was further partitioned with an *n*-hexane and 90 % methanol mixture. On the guidance of the constructed bioassay, the *n*-hexane soluble portion (28 g) was fractionated by silica gel column chromatography (*n*-hexane - EtOAc) to give 7 fractions (Fr. A1-Fr. A7). The Fr. A3

(360 mg) was further purified by HPLC (COSMOSIL 5C18-AR, MeOH-H_2O) to afford dictyoceratin-C (**1**) (36 mg). Dictyoceratin-C (**1**) was identified by ESI-TOF-MS and 2D-NMR analysis and comparison with authentic spectral data. Furospinosulin-1 (65 mg) was isolated from an active fraction of Fr. A1 (317 mg).

7. Aoki, S.; Kong, D.; Matsui, K.; Rachmat, R.; Kobayashi, M. *Chem. Pharm. Bull.* **2004**, *52*, 935.
8. Suna, H.; Arai, M.; Tsubotani, Y.; Hayashi, A.; Setiawan, A.; Kobayashi, M. *Bioorg. Med. Chem.* **2009**, *17*, 3968.
9. Semenza, G. L. *Physiology* **2009**, *24*, 97.
10. Semenza, G. L. *Oncogene* **2010**, *29*, 625.
11. The 40% confluent of DU145 cells in a 6-well plate were pre-incubated for 12 h in a humidified atmosphere of 5% CO_2 at 37 ºC (normoxic condition) or of 94% nitrogen, 5% CO_2, and 1% O_2 (hypoxic condition). The cells were then treated with the indicated concentrations of the testing compound for 24 h under normoxic or hypoxic conditions. Each culture medium was transferred to a new test tube and incubated at 4 °C for 12 h in the presence of anti-VEGF antibody to detect VEGF. Then, protein G agarose beads (100 μL) were added and incubated for another 2 h at 4 °C. After removal of the medium by centrifugation, the beads were washed 4 times with lysis buffer [10 mM Tris-HCl (pH7.5) containing 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM NaF, 2 mM sodium vanadate, 1% SDS, 1% protease inhibitor cocktail-DMSO solution, and 1% phosphatase inhibitor cocktail-DMSO solution] and boiled in the 2x sample buffer (100 mM Tris-HCl buffer pH 6.8, 4% SDS, 10% β-mercaptoethanol, 20% glycerol, 0.005% bromophenol blue) for 5 min. To detect HIF-1α and β-actin, the cells were rinsed

with ice-cold PBS and lysed in lysis buffer. The immunoprecipitates from the culture medium and the cell lysates were subjected to SDS-PAGE and transferred onto PVDF membranes. The membranes were probed with the appropriate primary antibodies. Immunopositive bands were visualized by using an ECL kit (GE Healthcare, Buckingham-shire, England).

12. The sesquiterpene phenols, dictyoceratin-C (**1**) and smenospondiol (**2**), did not show differentiation-inducing activity in human chronic myelogenous leukemia K562 cells up to 22.5 μM and 15 μM, respectively.

Figure legends

Figure 1. Chemical structures of compounds **1**-**10**.

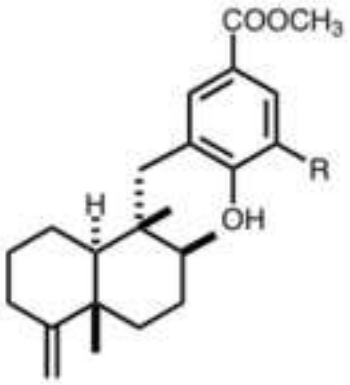
Figure 2. The anti-proliferative activity of compounds **1**-**10** against DU145 cells under both normoxic and hypoxic conditions.

DU145 cells (1×10^4 cells/well/200 μL) in a 96-well plate were pre-incubated for 12 h under normoxic or hypoxic conditions. The cells were then treated with the indicated concentrations of each compound for 24 h under the normoxic condition (closed bar) or the hypoxic condition (open bar). The growth inhibition rate was calculated as the percentage of parallel negative controls.

Figure 3. Effects of dictyoceratin-C (**1**) and dysideamine (**9**) on the accumulation of HIF-1α and the production of VEGF.

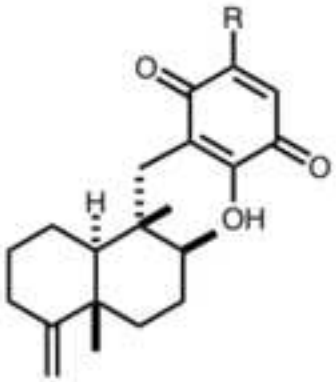
DU145 cells in the 6-well plate were incubated for 24 h under normoxic or hypoxic conditions in the presence or absence of the indicated concentrations of testing compound. VEGF in the culture medium was detected by immuno-precipitation with anti-VEGF antibody. To detect HIF-1α and β-actin, the cell lysate was resolved by

using SDS-PAGE and was incubated with anti-HIF-1α antibody or anti-β-actin antibody.



dictyoceratin-C (**1**) R = H

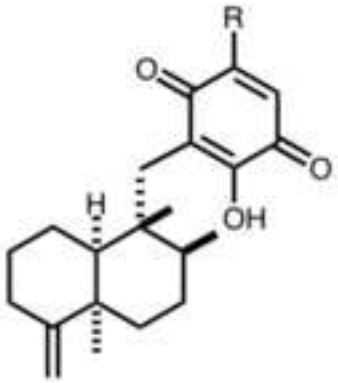
smenospondiol (**2**) R = OH



smenospongine (**3**) R = NH_2

smenospongorine (**4**) R = $NHCH_2CH(CH_3)_2$

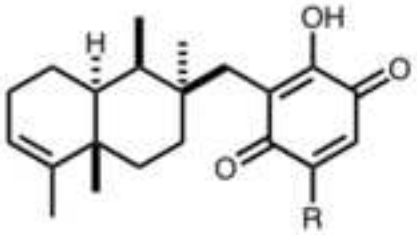
ilimaquinone (**5**) R = OCH_3



5-*epi*-smenospongine (**6**) R = NH_2

5-*epi*-smenospongorine (**7**) R = $NHCH_2CH(CH_3)_2$

5-*epi*-smenospongidine (**8**) R = $NHCH_2CH_2Ph$



dysideamine (**9**) R = NH_2

bolinaquinone (**10**) R = OCH_3

Figure 1

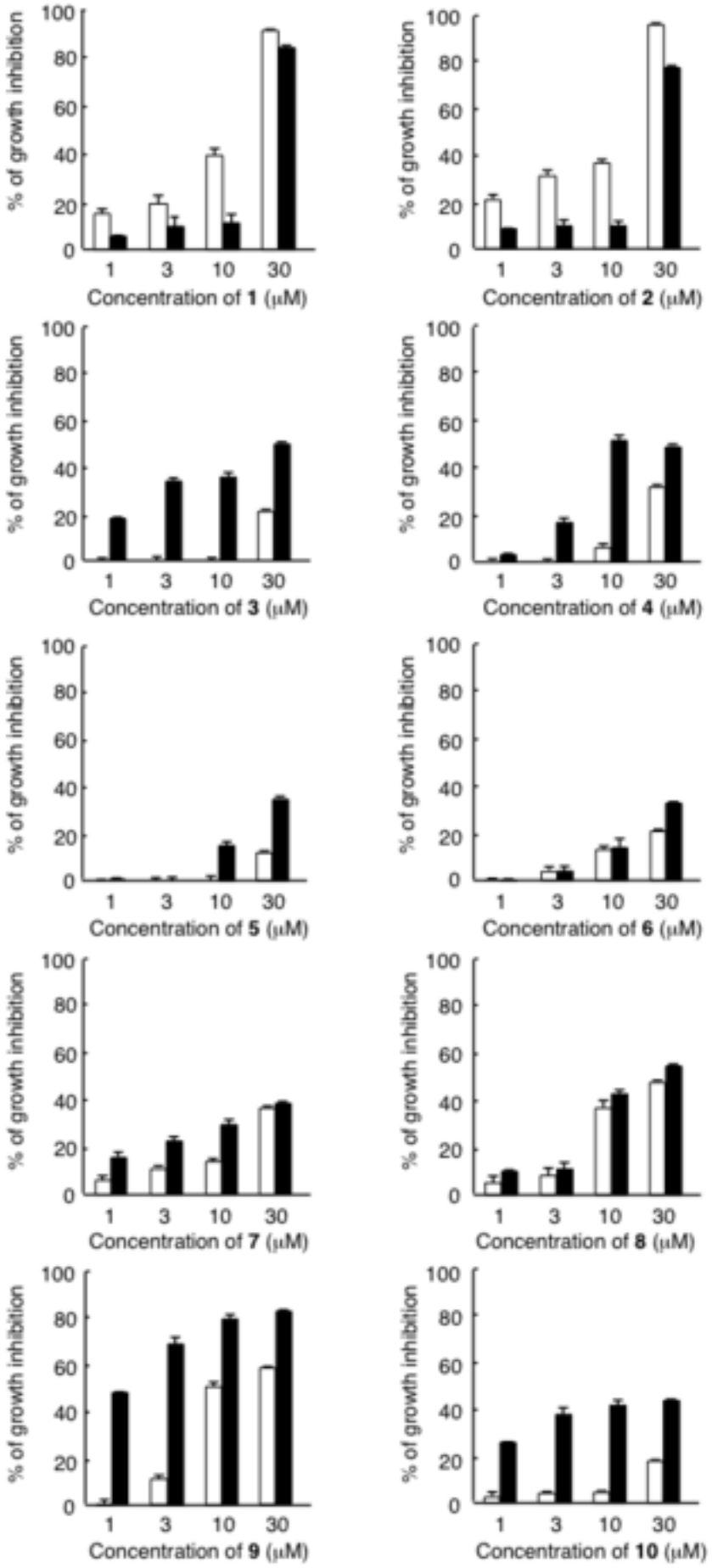

% of growth inhibition
100
80
60
40
20
0
1
3
10
30
Concentration of 1 (μM)
Concentration of 2 (μM)
Concentration of 3 (μM)
Concentration of 4 (μM)
Concentration of 5 (μM)
Concentration of 6 (μM)
Concentration of 7 (μM)
Concentration of 8 (μM)
Concentration of 9 (μM)
Concentration of 10 (μM)

Figure 2

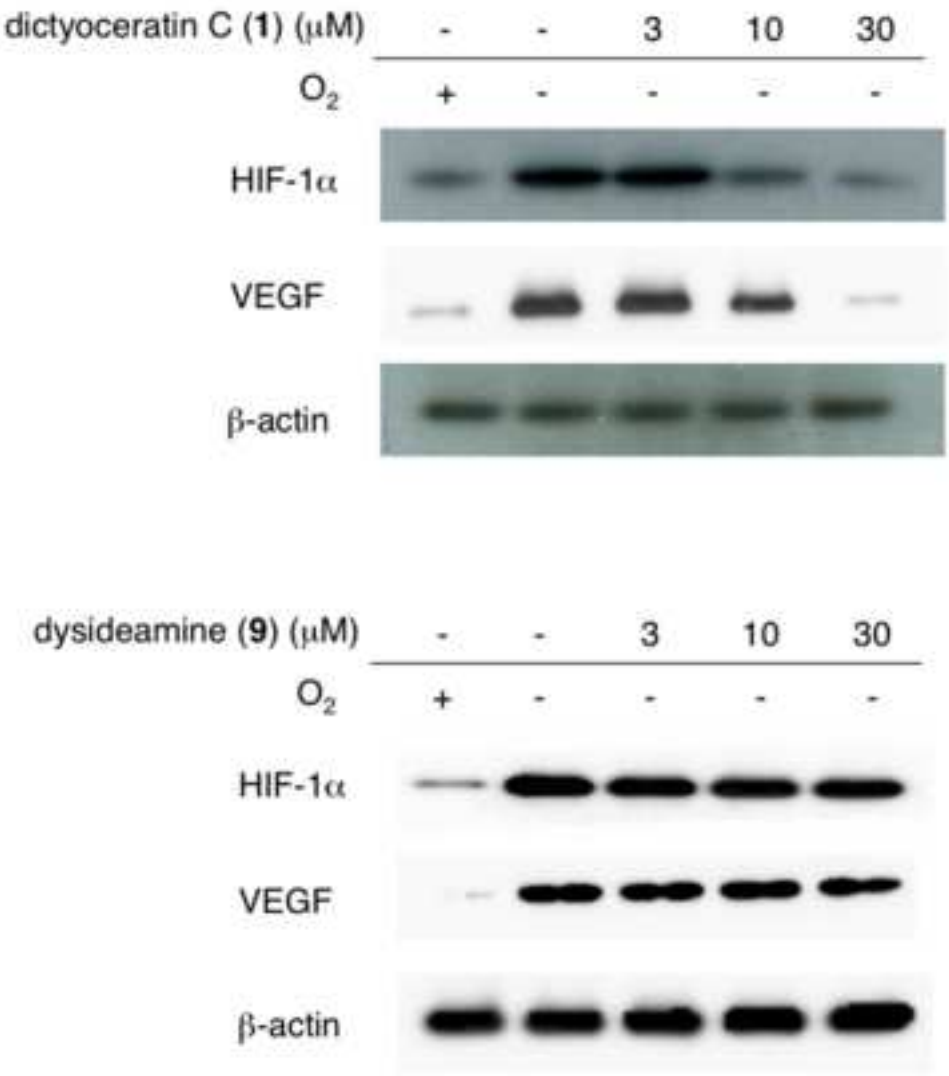


Figure 3

Marine spongian sesquiterpene hydroquinones, dictyoceratin-C and smenospondiol, display hypoxia-selective growth inhibition against cancer cells

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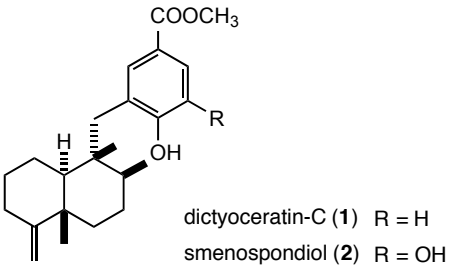
Marine spongian sesquiterpene hydroquinones, dictyoceratin-C (**1**) and smenospondiol (**2**), selectively inhibited proliferation of cancer cells under hypoxic condition, through preventing accumulation of Hypoxia-Inducible Factor-1α.



dictyoceratin-C (**1**) R = H

smenospondiol (**2**) R = OH