Sesquiterpene Aminoquinones, from a Marine Sponge, Induce Erythroid Differentiation in Human Chronic Myelogenous Leukemia, K562 Cells

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A new sesquiterpene aminoquinone, 5-epi-smenospongorine, together with nine known sesquiterpene quinone/phenols, was isolated as differentiation-inducing substances to K562 cells into erythroblast from the marine sponge Dactylospongia elegans. The structure–activity relationship study of these compounds clarified that the quinone skeleton is indispensable and the amino group plays an important role for their differentiation-inducing activity to K562 cells into erythroblast.

Key words 5-epi-smenospongorine; erythroid differentiation; structure–activity relationship; K562 cells; marine sponge

Despite the various cancer therapeutic methods such as chemotherapy, which are being carried out clinically, cancer remains to be a serious and, in most cases, fatal disease. Chemotherapeutic drugs affect not only tumor cells but also proliferative normal cells such as bone marrow cells and result in serious side effects. To solve this problem, several other alternative therapeutic methods are being developed. The differentiation-induction therapy, in which tumor cells are differentiated to normal cells, seems to be one promising therapy. As a good example of differentiation inducer, ATRA (all-trans-retinoic acid) has shown superior therapeutic effect as a differentiation inducer, ATRA (all-trans-retinoic acid) has shown superior therapeutic effect on acute promyelocytic leukemia (APL), which is unresponsive to conventional chemotherapeutic drugs. However, several kinds of leukemia such as human chronic myelogenous leukemia (CML) are unresponsive to ATRA. So, development of new differentiation inducers has been required.

As a part of our exploratory research of bioactive substances from marine organisms, we have been searching for new differentiation inducers to tumor cells and have found several inducers to ATRA-nonresponsive CML (K562 cells) or murine neuroblastoma (Neuro 2A cells) from marine sponges.2–4 Recently, we have isolated from a marine sponge2 a sesquiterpene aminoquinone, smenospongorine (4), which induces differentiation of K562 cells into erythroblasts. Study on its action mechanism showed that smenospongorine induced arrest of the cell cycle at the G1 phase and increased expression of p21 protein, a Cip1/Waf1 cdk (cyclin dependent kinase) inhibitor. This interesting action of smenospongorine led us to continue further study, and we isolated ten sesquiterpene quinone/phenol analogues from the same marine sponge, including a new compound named 5-epi-smenospongorine (1). In this paper, the structure–activity relationship of these sesquiterpene quinones is discussed.

Results and Discussion

Chemical Structures of 5-epi-Smenospongorine (1) and the Analogues The nine sesquiterpene quinone/phenols (2–10) together with a new compound named 5-epi-smenospongorine (1) were isolated from the marine sponge Dactylospongia elegans. (Fig. 1) Compounds 2–10 were identified as smenospongorine, 6,7 5-epi-smenospongorine, 6,8 smenospongorine,6,7 5-epi-smenospondiol, 6,7 ilimaquinone,6 ilimaquinone,7 dictyoceratin-
**epi-Smenospongorine and the Related Compounds**

The differentiation-inducing activities of these compounds were evaluated by the induction of hemoglobin production in K562 cells. Thus, pseudo-peroxidase activity of hemoglobin was measured by the colorimetric assay using diaminofluorene. The lowest concentrations of each compound (1—8) indicating similar differentiation-inducing activity are shown in Fig. 2, respectively. And, the highest concentrations showing no cytotoxicity were chosen for the two inactive sesquiterpene phenols, 9 and 10. Firstly, each of six sesquiterpene aminquinones (1—6) exhibited similar differentiation-inducing activity, which is stronger than that of aphidicolin, a well-known differentiation-inducing agent of K562 cells into erythroblasts. Secondly, compounds 9 and 10 does not have quinone function, showed no activity. Thirdly, compounds 7 and 8 showed differentiation-inducing activities at the higher concentration than those of 1—6. On the basis of these structure–activity relationship studies, the following evidences were obtained. 1) The quinone structure is indispensable. 2) The amino group should play an important role. 3) The substituents at the amino group are not crucial. 4) The configuration at the C-5 in sesquiterpene part is not important.

So far, these sesquiterpene quinone/phenols have been reported to exhibit anti-microbial, anti-HIV, anti-malarial, and cytotoxic activities. In this study, we clarified that these sesquiterpene aminoquinones exhibit differentiation-inducing activity to K562 cells into erythroblasts. The clinical application of Glivec (a synthetic compound targeting Bcr-Abl (breakpoint cluster region-Abelson leukemia sequence), 14) is well-known differentiation-inducing agent of K562 cells into erythroblasts. Secondly, compounds 9 and 10 do not have quinone function, showed no activity. Thirdly, compounds 7 and 8 showed differentiation-inducing activities at the higher concentration than those of 1—6. On the basis of these structure–activity relationship studies, the following evidences were obtained. 1) The quinone structure is indispensable. 2) The amino group should play an important role. 3) The substituents at the amino group are not crucial. 4) The configuration at the C-5 in sesquiterpene part is not important.

### Experimental

**Materials**

Marine sponge *Dactylospongia elegans* was collected at West Flores, Indonesia in 2001. 2,7-Diaminofluorene and aphidicolin were purchased from Wako Pure Chemical Industries Co. Ltd.

**Cell Lines**

Human chronic myelogenous leukemia cell line K562 was provided by RIKEN Cell Bank and routinely maintained in RPMI medium supplemented with 10% fetal bovine serum, 100 μg/ml kanamycin, and 0.44 mg/ml glutamine at 37 °C in a humidified atmosphere of 5% CO₂.

**Extraction and Isolation**

The frozen marine sponge (2.6 kg, wet weight) was cut into pieces and soaked in methanol overnight. The resulting methanol, n-BuOH, and H₂O phases. The 90% methanol phase was evaporated in vacuo followed by precipitation with diethylamine to obtain 5-Smenospongorine. The inactive Fr. A and Fr. B were also separated by ODS column (MeOH–H₂O) or HPLC (Cosmosil 55L, hexane–AcOEt–acetone–MeOH) to give three fractions (Fr. A (0.42 g), Fr. B (0.73 g), Fr. C (0.83 g)). The active Fr. C was further separated by ODS column (Cosmosil ODS, MeOH–H₂O) and reversed-phase HPLC (CAPCELL PAK C18 UG120, 70% aq. MeOH–0.1% diethylamine) to obtain 1 (3 mg, 0.15% from the 90% MeOH extract), 2 (5 mg, 0.25%), 3 (19 mg, 0.95%), 4 (9 mg, 0.45%), 5 (3 mg, 0.15%), 6 (4 mg, 0.20%), 7 (5 mg, 0.25%), 8 (3 mg, 0.15%). The inactive Fr. A and Fr. B were also separated by ODS column (MeOH–H₂O or HPLC (Cosmosil 55L, hexane–AcOEt=7:1) to obtain 9 (20 mg, 1%) and 10 (17 mg, 0.85%).

**1H- and 13C-NMR Data for 5-epi-Smenospongorine (1)**

<table>
<thead>
<tr>
<th>No.</th>
<th>δC (ppm)</th>
<th>δ (mult., J/Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.5</td>
<td>2.13 (m)</td>
</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>1.77 (m)</td>
</tr>
<tr>
<td>3</td>
<td>32.9</td>
<td>2.43 (d, 13.8)</td>
</tr>
<tr>
<td>4</td>
<td>153.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>39.5</td>
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</tr>
<tr>
<td>6</td>
<td>44.4</td>
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<td>7</td>
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</tr>
<tr>
<td>10</td>
<td>48.4</td>
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</table>

*a* 125 MHz in CDCl₃,  *b* 500 MHz in CDCl₃,  *c* Interchangable.

**Induction of Hemoglobin Production in K562 Cells**

For the evaluation of hemoglobin production in K562 cells, pseudo-peroxidase activity of hemoglobin was measured by the colorimetric assay using diaminofluorene. The suspension of K562 cells (4 × 10⁴ cells/ml) was placed in a 24-well plate. After addition of 10 μl of the DMSO solution of the testing sample, the culture was incubated at 37 °C under 5% CO₂ atmosphere for 4 d. The culture medium was centrifuged at 3000 × g for 3 min, and the resulting supernatant was removed. After addition of 50 μl of ultra-pure water, the
lysed cells were treated with 100 μl of the diaminofluorene solution [5 mg of diaminofluorene was dissolved in 1 ml of the 90% acetic acid and Tris–HCl buffer (pH 7.5) (10:1) solution] and 10 μl of 3% hydrogen peroxide and then kept for 30 min in the dark. The absorbance at 630 nm was measured by a microplate reader. The drug-untreated cells were used as the negative control.

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