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Apoptosis-Inducing Activity of Synthetic Intermediates of Halichlorine

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Abstract—Synthetic intermediates of alkaloid halichlorine with the azaspiro core structure have been found to induce apoptosis of cultured human cells including an acute monocytic leukemia cell line (THP-1) at micromolar concentrations. The novel biological activity of the intermediates was suggested to depend on the skeletal structure and silyloxymethyl functionality on the five-membered ring.

Halichlorine[1] is an alkaloid isolated from the sponge *Halichondria okadai* Kadota in 1996 that possesses an azaspiro-[4,5]decane substructure (Fig. 1).

This substance was shown to inhibit the induced expression of VCAM-1 (vascular cell adhesion molecule-1)[2] in cultured human umbilical vein endothelial cells, and might be a potential target for the treatment of allergic inflammation, arteriosclerosis and cancer.[3] The unique structure and biological activity of the compound has promoted a variety of synthetic approaches[4] including total synthesis.[5] In this study, we analyzed the biological effects on human cultured cells of several compounds with a spirocyclic core subunit prepared in the course of total synthetic studies on halichlorine. Some of these compounds were unexpectedly found to exhibit apoptosis-inducing activity as a novel biological function.

Syntheses of Compounds with the Spirocyclic Core Structure of Halichlorine

For biological activity evaluation, five bicyclic compounds (**2–5**, **9**) and eleven tricyclic compounds (**6–8**, **10–17**) were prepared from tricyclic ester **1**, which was used as a key intermediate in our previous synthetic studies[4h] on halichlorine, as shown in Scheme 1.

Cytotoxic Activities of Azaspirobicyclic Intermediates

The cytotoxic activities of synthesized key intermediates **1–17** were assayed by the XTT method[5] with a cultured human monocytic leukemia cell line (THP-1).[6] Cell viability was colorimetrically estimated by conversion of the XTT reagent to its formazan derivative with the succinate tetrazolium reductase in the living cell culture system in RPMI1640 medium containing 10% fetal calf serum in the presence of 5% CO_2 at 37 °C Figure 2 shows the dose-dependency of the cytotoxic effects of the synthetic intermediates with the azaspirobicyclic structure on THP-1 cells when ethanol solutions of them were added to the culture medium. Compounds **6**, **9**, **15** and **16** induced dose-dependent decreases in cell viability, whereas **7**, **10** and **12** had lower effects in the same range.

Table 1 summarizes the concentrations necessary to decrease cell viability by 50% (IC_{50}). The values for **16**,

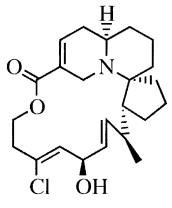

Figure 1. Halichlorine.

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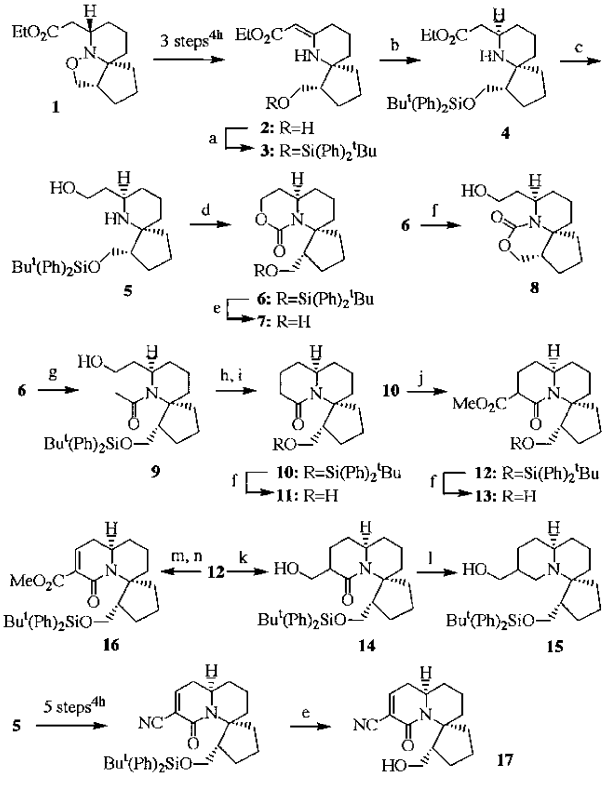
5, **9**, **6**, **15**, **4**, **14** and **10** were 5.0, 8.9, 25, 32, 32, 40, 63 and 71 μM, respectively. The other agents had lower cytotoxic effects at concentrations below 100 μM. From the aspect of the structure–activity relationship, the spirobicyclic core substructure, the protecting *t*-butyldiphenylsilyl (TBDPS) group at C14 and the distinct functional groups were considered to be responsible for the expression of the cytotoxic activity. Spirotricyclic **16** exhibited the most potent effect among the compounds, having a substructure similar to that of the natural product. Interestingly, **12**, a derivative lacking a double bond (C2–C3), exhibited little cytotoxic activity.

Induction of Chromatin Condensation by the Cytotoxic Intermediates

We also examined the effects of the cytotoxic intermediates on induction of chromatin condensation as one of the apoptotic phenomena in THP-1 cells. Vital staining with Hoechst 33258[7] for 2 h was performed after treatment with the compounds for 4 h. From the fluorescent microscopic images shown in Figure 3, cytotoxic **16** and **4** were found to induce chromatin condensation at the concentrations of 5 and 40 μM, respectively. Twenty micromolar **6** and **15** also caused remarkable condensation (data not shown). In contrast, non-toxic intermediates including **3** and **12** did not induce chromatin condensation at all. In the case of potent cytotoxic **5**, chromatin condensation was hardly observed, although considerable cell debris was detected under the experimental conditions (data not shown). Partial induction by the other cytotoxic compounds was observed when they were added at concentrations above 50 μM.



Scheme 1. (a) $^{t}Bu(Ph)_2SiCl$, imidazole, 4-DMAP, CH_2Cl_2, rt, quant.; (b) H_2, PtO_2, EtOH, rt, quant.; (c) $LiAlH_4$, THF, rt, quant.; (d) triphosgene, Et_3N, CH_2Cl_2, rt, quant.; (e) HF·pyridine, THF, rt, 81% for **7**, 63% for **17**; (f) $^{n}Bu_4NF$, THF, 50 °C, 76% for **8**, 89% for **11**, 68% for **13**; (g) MeLi, $BF_3{\cdot}OEt_2$, Et_2O, −78 °C, quant.; (h) I_2, Ph_3P, imidazole, benzene, rt, 95%; (I) LDA, THF, −78 °C, 95%; (j) LDA, $ClCO_2Me$, THF, −78 °C, quant.; (k) $LiBH_4$, Et_2O, MeOH, reflux, 82%; (l) BH_3·THF, THF, rt, 87%; (m) LDA, PhSeCl, THF, rt; (n) *m*CPBA, CH_2Cl_2, 58% (two steps).

Activation of Caspase by the Cytotoxic Intermediates

We also examined whether or not intracellular activation of caspase 3[8] and/or 7,[9] as apoptotic executioners, could be induced by the addition of the cytotoxic intermediates. After treatment of THP-1 cells with the compounds for a definite period, cell extracts were prepared, and then caspase activity was measured fluorometrically with acetyl-DEVD-AMC[10,11] as a substrate. As shown in Figure 4, cytotoxic **16**, **15** and **4**, but not **5**, induced the activation of caspase time-dependently while **3** and **12**, which exhibit little cytotoxicity at the indicated concentrations, did not induce caspase 3 activation. These results strongly suggest that some of the key intermediates of halichlorine cause apoptosis of THP-1 cells. In contrast, such activation was not observed when cells were treated with potent cytotoxic **5**. One of the reasons for this is that the cell extract was hard to prepare because **5** induced the destruction of a large number of the cells immediately on treatment at 5 μM. It is also possible that the cytotoxic effect of **5** may be induced by a different mechanism from that of apoptosis.

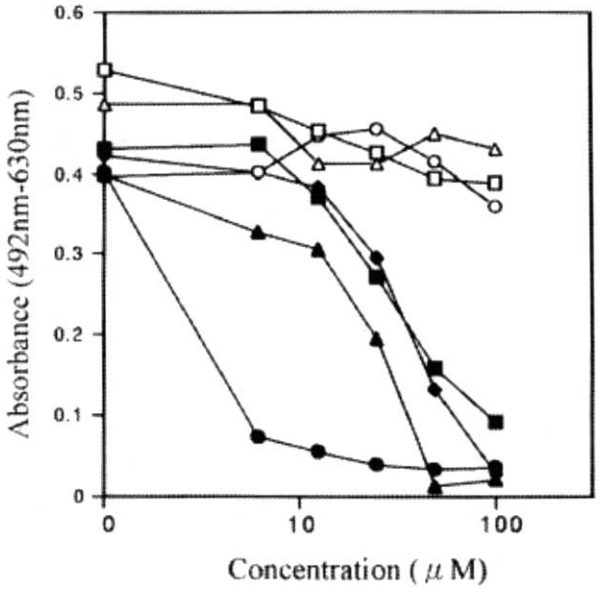


Figure 2. Cytotoxic activity of **6** (■), **9** (▲), **15** (◆), and **16** (●) towards the THP-1 cell line treated with the indicated concentrations for 4 h at 37 °C. Each value is the mean of two independent measurements with the XTT method. Compounds **7** (□), **10** (△), and **12** (○) did not exhibit the cytotoxic activity.

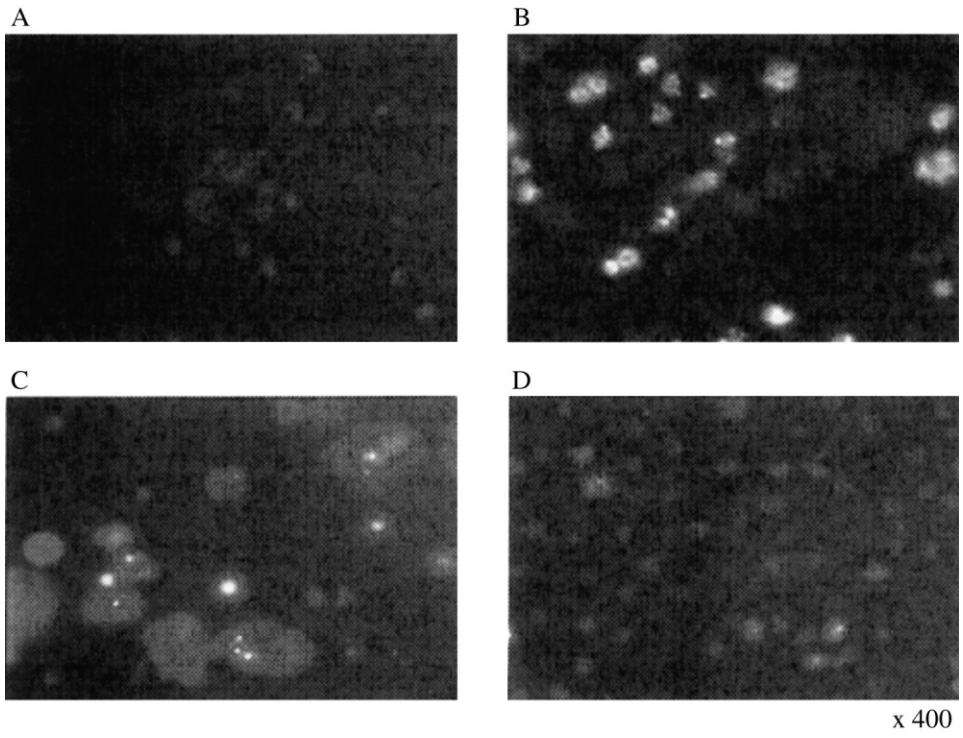


Figure 3. Induction of chromatin condensation in THP-1 cells by spirocyclic intermediates: (A) control (no agent); (B) 5 μM **16**; (C) 40 μM **4**; (D) 50 μM **3**. The cells were stained with Hoechst 33258 (0.2 μM) in culture after incubation with the compounds for 4 h at 37 °C. Original magnification, ×400.

Table 1. Cytotoxic activity of key intermediates **1**–**17** towards the THP-1 cell line in culture

Compd	IC_{50}, μM[a]
1	>100
2	>100
3	>100
4	40
5	8.9
6	32
7	>100
8	>100
9	25
10	71
11	>100
12	>100
13	>100
14	63
15	32
16	5.0
17	>100

[a]Values represent the concentrations of the compounds in the culture medium necessary to decrease the cell viability by 50%, that were determined from dose–response curves like those shown in Figure 2.

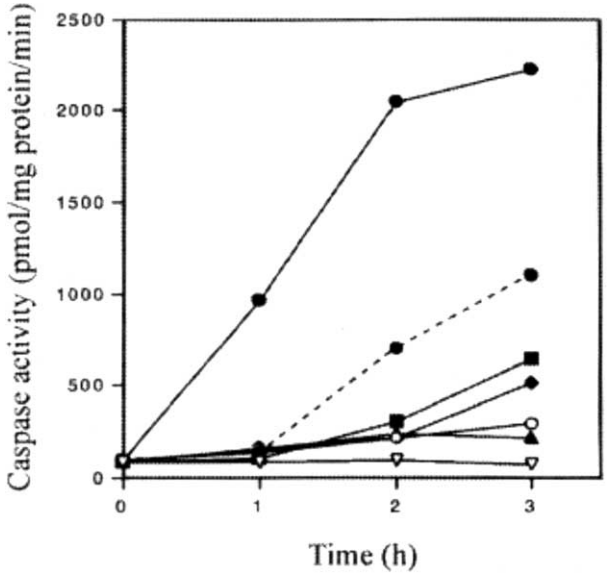


Figure 4. Time-dependent activation of caspase 3 and/or 7 in THP-1 cells by 5 μM **16** (–●–), 20 μM **16** (●), 20 μM **15** (◆) and 20 μM **4** (■) at 37 °C. 20 μM **3** (▽), 20 μM **12** (○) and 20 μM **5** (▲) did not induce caspase activation at all.

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