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Development of a Novel Inducer of Protein–Protein Interactions Based on Aplyronine A

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Takayuki Ohyoshi,[a] Atsuhiro Takano,[a] Mayu Namiki,[a] Tomotaka Ogura,[a] Yuto Miyazaki,[a] Yuta Ebihara,[a] Koichi Takeno,[a] Ichiro Hayakawa,[b*] Hideo Kigoshi[a*]

An aplyronine A–swinholide A hybrid, consisting of the macrolactone part of aplyronine A and the side chain part of swinholide A, was designed, synthesized, and evaluated for biological activities. The hybrid retained strong cytotoxicity and actin–depolymerizing activity. In addition, the hybrid induced protein–protein interactions (PPI) between actin and tubulin in the manner of aplyronine A.

Introduction

Natural products that induce PPI, such as FK-506[1] and rapamycin,[2] are considered to show unique and potent biological activities.[3] Aplyronine A (**1**),[4-6] a PPI inducer of marine origin, forms a 1 : 1 : 1 heterotrimeric complex with actin and tubulin, inhibits tubulin polymerization, and exhibits extremely potent antitumor activity (Figure 1).[7] From the results of our previous experiments, we deduced that aplyronine A (**1**) first binds with actin by the side chain portion and then interacts with the tubulin α,β-heterodimer through the trimethylserine group at the C7. Actin and tubulin are major cytoskeletal proteins, and modulators of their functions are expected to show significant bioactivities. For example, modulators of tubulin polymerization, such as vinca alkaloids, have been used as clinically important drugs.[8] Because aplyronine A (**1**) shows potent antitumor activities *in vivo* against P388 leukemia, Lewis lung carcinoma, and Ehrlich carcinoma,[9] aplyronine A (**1**) is expected to be a novel type of anticancer drug candidate based on PPI between actin and tubulin. Thus, we planned to develop a lead compound for an anticancer drug based on aplyronine A (**1**). To this end, we herein studied the structure-activity relationship of aplyronine A (**1**) based on its hybridization of natural products.

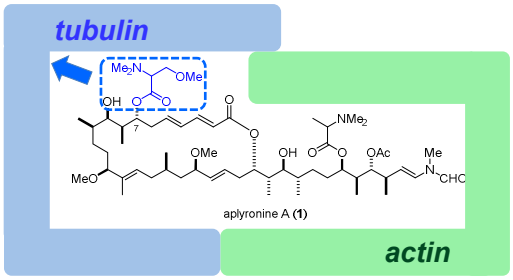

Figure 1 Structure of aplyronine A (**1**) and a pattern diagram of the heterotrimeric complex formed between aplyronine A (**1**), actin, and tubulin

Design of aplyronine A–swinholide A hybrid 4

The previous analysis of the relationship between the structure and actin-depolymerizing activity of aplyronine A (**1**) demonstrated that its side chain portion is crucial for its activity.[10] On the other hand, mycalolide B (**2**), whose side chain is similar to that of aplyronine A (**1**), interacts more strongly with actin[11] (K_d = 13–20 nM) than aplyronine A (**1**)[12] dose (K_d = 100 nM). From the above results, we designed and synthesized a hybrid **3** consisting of the macrolactone of aplyronine A (**1**) and the side chain of mycalolide B (**2**), and evaluated both its cytotoxicity against HeLa S3 cells and its actin-depolymerizing activity (Figure 2).[13] The results showed that the aplyronine A–mycalolide B hybrid **3** retained potent actin-depolymerizing activity (EC_{50} = 1.0 μM for 3 μM actin), but its cytotoxicity against HeLa S3 cells was considerably reduced (IC_{50} = 12 nM) compared to that of aplyronine A (**1**) (IC_{50} = 0.010 nM). We realized that the differences in the pattern and stereochemistry of substitution at C24–C25 between aplyronine A (**1**) and the aplyronine A–mycalolide B hybrid **3** significantly influenced their respective cytotoxicities. This consideration was supported by a comparison of the X-ray crystallographic structures between the actin–aplyronine A (**1**) complex[14] and an actin–kabiramide C (mycalolide B-related compound) complex[15]. Swinholide A (**5**), a cytotoxic

[a] Department of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8571, Japan
E-mail: kigoshi@chem.tsukuba.ac.jp
[b] Division of Applied Chemistry, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan
E-mail:ichiro.hayakawa@okayama-u.ac.jp
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macrolide[16] isolated from the marine sponge *Theonella swinhoei*,[17] shows actin-depolymerizing activity. The K_d value of swinholide A (**5**) in complex with actin is almost the same as that of aplyronine A (**1**) in complex with actin.[18] Rayment et al. reported the X-ray crystallographic analysis of an actin–swinholide A (**5**) complex,[19] revealing that the side chain portion of swinholide A (**5**) interacted with actin in the same way as that of aplyronine A (**1**).

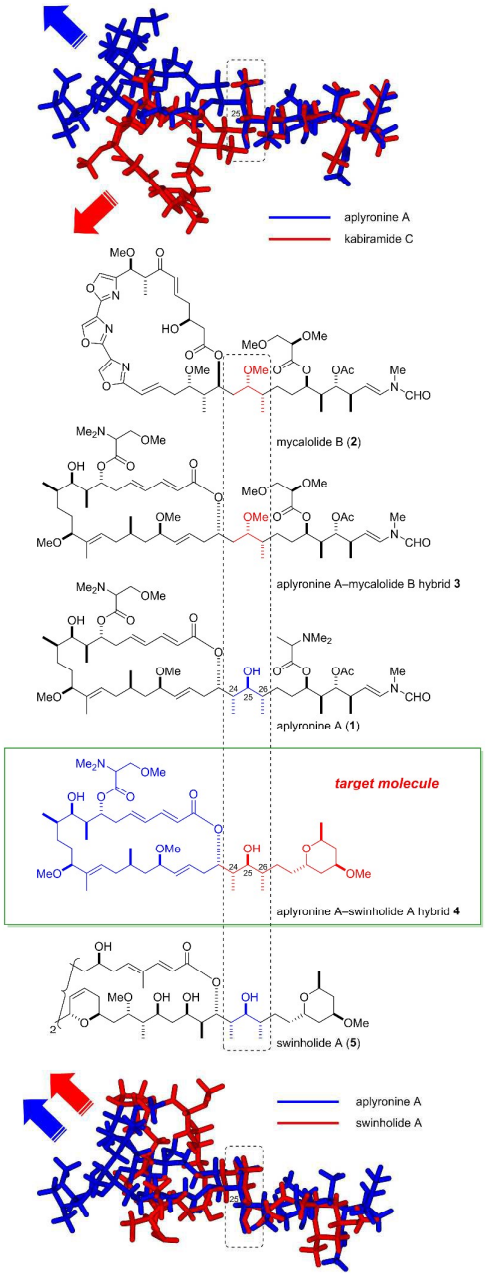
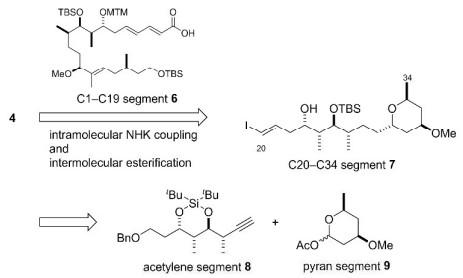


Figure 2 Structures of actin-depolymerizing natural products and their hybrid and superimposing conformations of actin–aplyronine A and –kabiramide C or –swinholide A complexes based on X-ray analyses. The arrows indicate the orientation of the macrolactone part on the corresponding complexes.

Superimposed conformations of actin–aplyronine A and –kabiramide C or –swinholide A complexes based on X-ray analyses are shown in Figure 2. The differences in configuration at C25 and the degree of substitution at C24 between aplyronine A and kabiramide C cause a change in the conformational relationship between the macrolactone and the side chain part. On the other hand, because the stereochemistry of C25 and the degree of substitution at the C24–C26 are same between aplyronine A and swinholide A, the macrolactone part of swinholide A would correspond with those of aplyronine A on their actin complexes. Hence, we designed aplyronine A–swinholide A hybrid **4**. Because the stereochemistry and the degree of substitution at C24–C26 of swinholide A (**5**) are coincident with those of aplyronine A (**1**), we expected that the conformation of the aplyronine A–swinholide A hybrid **4** would be similar to that of aplyronine A (**1**). Consequently, we presumed that aplyronine A–swinholide A hybrid **4** showed potent cytotoxicity by the same mechanism as aplyronine A (**1**). Also, the simpler structure of the side chain portion of swinholide A (**5**) may make aplyronine A–swinholide A hybrid **4** a synthetically accessible analog that still exhibits the biological activity of aplyronine A (**1**). Thus, we planned to synthesize aplyronine A–swinholide A hybrid **4**.

Synthesis of aplyronine A–swinholide A hybrid 4

The retrosynthetic pathway of aplyronine A–swinholide A hybrid **4** is shown in Scheme 1. Thus, we planned the synthesis of hybrid **4** based on our second-generation total synthesis of aplyronine A (**1**).[5c] Hybrid **4** was assembled from C1–C19 segment **6**[20] and C20–C34 segment **7** by using intermolecular esterification and the intramolecular Nozaki–Hiyama–Takai–Kishi (NHK) coupling[21] reaction. C20–C34 segment **7** might be obtained from acetylene segment **8** and known pyran segment **9** by Miyashita's method with modification.[22]



Scheme 1 Retrosynthetic pathway of aplyronine A–swinholide A hybrid **4**

The two segments[23] **8** and **9** were converted into aplyronine A–swinholide A hybrid **4** as follows (Scheme 2). Thus, the coupling reaction between **8** and **9** gave a coupling compound,[22] which was converted into alcohol **10** through a catalytic reduction for the triple bond and benzyl group. Oxidation of alcohol **10** with Dess-Martin periodinane and Takai olefination of the resultant aldehyde afforded iodoolefin **11**.[24] Then, we manipulated the protecting groups in iodoolefin **11** to give C20–C34 segment **7** in four steps: (1) removal of the silylene acetal, (2) regioselective pivalation at 23-*O*, (3) TBS

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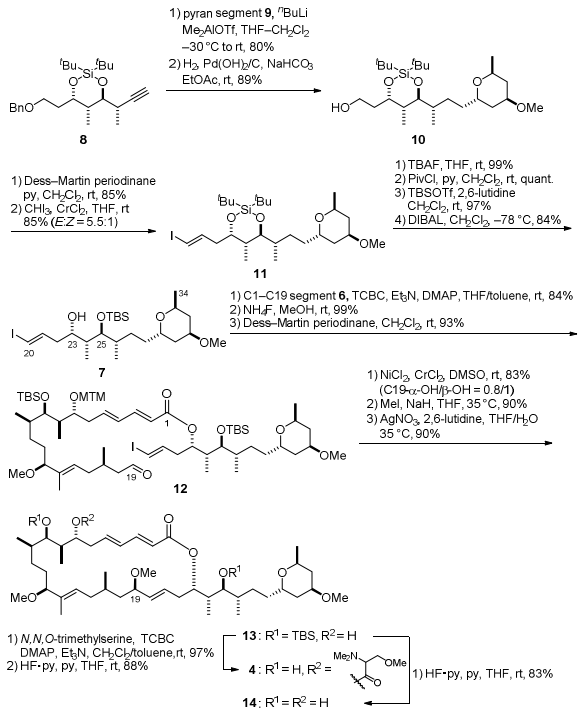
protection of the remaining hydroxy group, and (4) reductive removal of the pivaloyl group. The connection of C1–C19 segment **6**[20] and C20–C34 segment **7** was achieved by Yamaguchi esterification in 84% yield. Removal of the primary TBS group followed by Dess–Martin oxidation produced NHK coupling precursor **12**. Intramolecular NHK coupling of **12** gave the desired macrolide and the C19 diastereomer, which could be separated by silica gel chromatography.[25] Methylation of the resulting hydroxy group and removal of the MTM group gave alcohol **13**. Finally, introduction of the *N,N,O*-trimethylserine ester group and removal of two TBS groups afforded aplyronine A–swinholide A hybrid **4**. The synthesis of hybrid **4** was 16 steps shorter than the 86-step synthesis of aplyronine A. In addition, to confirm the mode of action of hybrid **4**, aplyronine C (a natural aplyronine derivative lacking the trimethylserine ester group)–swinholide A hybrid **14** was also synthesized.



Scheme 2 Syntheses of aplyronine A–swinholide A hybrid **4** and aplyronine C–swinholide A hybrid **14**

Biological activities of the hybrid 4 and 14

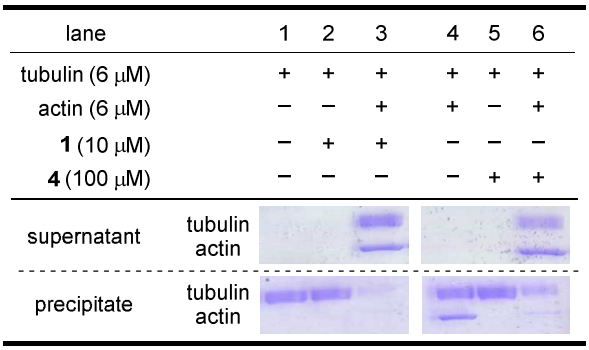
The cytotoxicities of **1**, **3**, **4**, and **14** against HeLa S3 cells were evaluated (Table 1). Hybrid **4** had strong cytotoxicity and actin-depolymerizing activity, but these activities were somewhat weaker than those of aplyronine A presumably due to the simplification of the side chain. On the other hand, hybrid **4** was found to have about 10000-fold stronger cytotoxicity than hybrid **14** (aplyronine C–swinholide A hybrid **14** : IC_{50} = 1500 nM). This fact indicates a similar tendency with aplyronines A and C, where the trimethylserine moiety is very important for cytotoxicity. In addition, the cytotoxicity of **4** was stronger than that of aplyronine A–mycalolide B hybrid **3**. Hybrid **3** was designed in our previous work (aplyronine A–mycalolide B hybrid **3** : IC_{50} = 12 nM), where we showed that the stereochemistry at C24–C25 was very important for cytotoxicity.

Table 1 Cytotoxicity and actin-depolymerizing activity.

compound	cytotoxicity against HeLa S3 cells IC_{50} (nM)[a]	actin depolymerizing activity EC_{50} (μM)[a]
1	0.01	1.3
3	12[b]	1.0[b]
4	0.17	12.8
14	1500	–

a. For experimental details, see ESI
b. ref. 13

Finally, since hybrid **4** retained strong cytotoxicity, we examined whether it would induce PPI between actin and tubulin (Figure 3). When tubulin was treated with aplyronine A (**1**) alone, tubulin was detected in the precipitate (lane 2). In lane 3, when actin was added to the condition of lane 2, actin and most of the tubulin were detected in the supernatant. Actin and tubulin did not interacted directly with each other, and polymerized proteins were detected (lane 4). These results confirmed that these experiments are suitable for detecting a PPI inducing ability of aplyronine A (**1**) between actin and tubulin, resulting in depolymerization of actin and tubulin. Hybrid **4** showed the same result as aplyronine A (**1**) (lanes 5 and 6). Therefore, hybrid **4** induces PPI between actin and tubulin just as does **1**, albeit with 10% of the potency of **1**. This results support our hypothesis that aplyronine A (**1**) binds to actin with its side chain moiety and the actin–aplyronine A (**1**) complex interacts with tubulin by using the macrolactone moiety with the trimethylserine to give actin–tubulin–



aplyronine A (**1**) complex, resulting in depolymerization of actin and tubulin.

Figure 3 *In vitro* microtubule depolymerization assay. Tubulin was polymerized with taxol (2 μM) in the presence of actin and/or **1** or **4**, and then precipitated by ultracentrifugation. Proteins in the supernatant and the precipitate were analyzed by SDS-PAGE, and detected with CBB stain. Polymerized protein was detected in the precipitate fraction, while

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depolymerized protein was detected in the supernatant fraction.

Conclusion

We designed and synthesized aplyronine A–swinholide A hybrid **4**, which consists of the macrolactone of aplyronine A and the side chain of swinholide A. Hybrid **4** retained strong cytotoxicity and actin-depolymerizing activity. In addition, hybrid **4** induces PPI between actin and tubulin in the manner of aplyronine A. Development of simplified hybrids for a new type of antitumor drug is ongoing in our laboratory.

Acknowledgement

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