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Synthesis of actin-depolymerizing compounds

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ABSTRACT

The artificial actin-depolymerizing compounds **3–6**, based on aplyronine A, an actin-depolymerizing antitumor marine macrolide, were synthesized, and their actin-depolymerizing activities and cytotoxicities were evaluated.

Actin-disrupting marine natural products are of interest to natural products chemists and pharmacologists.[1] Aplyronine A (**1**), an antitumor macrolide isolated from *Aplysia kurodai*,[2] interacts with actin, the major protein in cytoskeleton. Actin regulates various cell functions such as muscle contraction, cell motility, and cell division. Actin exists as a dynamic equilibrium mixture of two forms; polymeric F-actin and monomeric G-actin. Aplyronine A (**1**) not only inhibits the polymerization of actin by sequestering G-actin and forming a 1:1 complex, but also depolymerizes F-actin to G-actin by severing.[3] We investigated the structure-activity relationships of aplyronine A (**1**) using natural and synthetic analogs: the side-chain in **1** is essential for actin-depolymerizing activity, and analog **2**, which consists only of the side-chain moiety of **1**, exhibits strong activity (Fig. 1).[4] Structure-activity relationships of bistramide A[5] and iejimalides[6] were investigated in detail. We recently determined the crystal structure of actin-aplyronine A complex via synchrotron X-ray analysis[7] and obtained chemical evidence for the direct interaction between actin and the side-chain portion of aplyronine A by photoaffinity labeling experiments.[8] These results demonstrate the great importance of the side-chain moiety in the activity against actin. To obtain further information regarding the importance of the side-chain, we planned to synthesize short side-chain analogs **3–6**. The phenyl group in **4–6** is expected to enhance the affinity for actin through hydrophobic interaction and/or CH-π interaction. In this paper, we report the synthesis of **3–6** and their actin-depolymerizing activities and cytotoxicities against HeLa S_3 cells.

The synthesis of **3** started from alcohol **7**[4d,e], a synthetic intermediate of aplyronine A (**1**) (Scheme 1). Oxidation of **7** with TEMPO and $PhI(OAc)_2$[9] followed by a Takai olefination reaction[10] gave vinyl iodide **8**. Vinyl iodide **8** was subjected to a Buchwald amidation reaction[11] to afford enamide **9**, a common intermediate of analogs **3–6**. Removal of the acetonide protecting group of **9** and regioselective acylation gave analog **3**.[12]

Analogs **4–6** with a phenyl group were synthesized from enamide **9**. Regioselective cross olefin metathesis reaction of **9** with styrene, 4-phenyl-1-butene, and 6-phenyl-1-hexene provided olefins **10**, **11**, and **12**, respectively. While olefins **10** and **12** were obtained in good yields (82% and 86%, respectively), olefin **11** was obtained in only 48% yield due to isomerization of the double bond into byproduct **13**.

The actin-depolymerizing activities and cytotoxicities against HeLa S_3 cells of analogs **3–6** are summarized in Table 1. Analog **3** with enamide and dimethylalanine group, which corresponds to the half-length side-chain of aplyronine A (**1**), showed actin-depolymerizing activity, although this activity was weak. This result corresponds to X-ray analysis of actin-aplyronine A complex: both enamide and dimethylalanine groups of aplyronine A (**1**) interacted with actin by hydrogen bonds.[7] Analogs **4–6** with a phenyl group showed stronger actin-depolymerizing activity than **3**. These results indicate that (1) the terminal portion of the side-chain plays a key role in its activity, and (2) the phenyl group enhances the activity: hydrophobic and/or aromatic nature is important. Analogs

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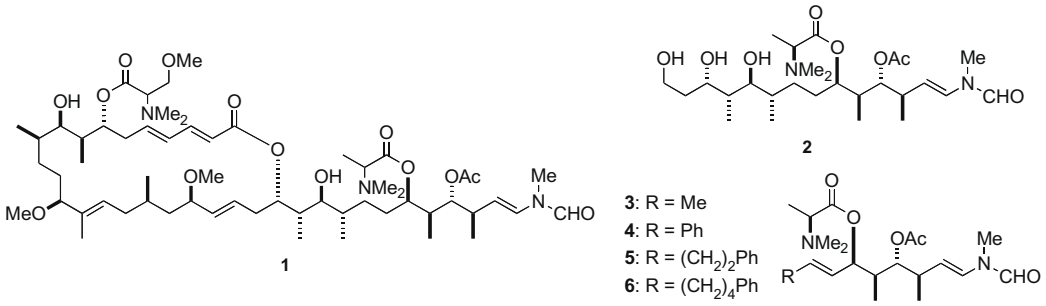
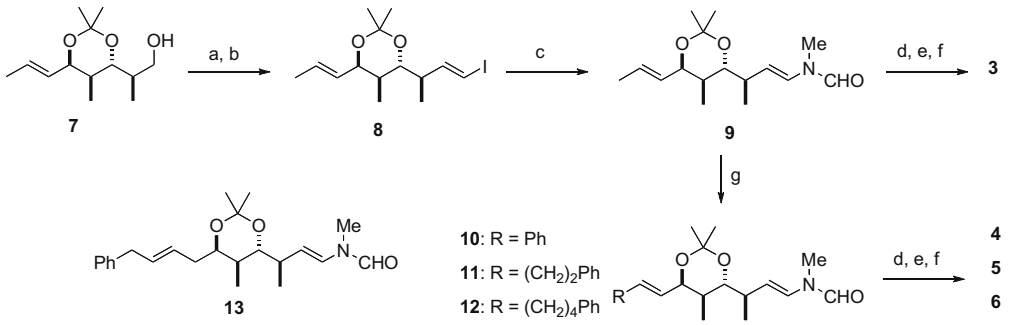


Figure 1. Structures of aplyronine A (**1**) and its artificial side-chain analogs.



Scheme 1. Reagents and conditions: (a) TEMPO, $PhI(OAc)_2$, CH_2Cl_2, rt; (b) $CrCl_2$, CHI_3, THF, 0 °C, 75% (2 steps); (c) CuI, K_3PO_4, MeNHCHO, *trans*-1,2-cyclohexanediamine, 60 °C, 93%; (d) PPTS, MeOH, 100% (**3**), 91% (**4**), 100% (**5**), 96% (**6**); (e) L-*N*,*N*-dimethylalanine, DCC, CSA, DMAP, rt 83% [**3**, based on recovered starting material (br sm)], 47% (**4**), 84% (**5**, br sm), 81% (**6**, br sm); (f) Ac_2O, pyridine, DMAP, rt, 96% (**3**), 100% (**4**), 81% (**5**), 100% (**6**); (g) 2nd Grubbs catalyst, RCH=CH_2, CH_2Cl_2, 40 °C, 82% (**10**), 48% (**11**), 20% (**13**), 86% (**12**).

Table 1
Actin-depolymerizing activities and cytotoxicities against HeLa S_3 cells of aplyronine A and the artificial analogs

Compounds	Actin-depolymerizing activity[a] IC_{50}, mM	Cytotoxicity IC_{50}, mg/mL
1	1.6[b]	0.00048[c]
2	7.9[b]	>10[b]
3	210	>10
4	85	>10
5	47	>10
6	21	>10

[a] Activity was monitored by measuring the fluorescent intensity of pyrenyl actin. For the assay conditions, see Ref. 4e. IC_{50} indicates the concentration required to depolymerize F-actin (3.7 mM) to 50% of its control amplitude.
[b] Ref. 4e.
[c] Ref. 4a.

3–6 exhibited no cytotoxicity against HeLa S_3 cells below 10 μg/mL. For analyzing cytotoxic mechanism of aplyronine A, cytotoxicities of aplyronine A (**1**) in the presence (5 μg/mL) or absence of analog **6** were evaluated. However, cytotoxicity of aplyronine A (**1**) was not inhibited by analog **6**.[13]

In conclusion, we synthesized the artificial actin-depolymerizing compounds **3–6**, based on aplyronine A, an actin-depolymerizing antitumor marine macrolide, and their actin-depolymerizing activities and cytotoxicities were evaluated. Among the synthesized analogs, compound **6** showed relatively strong actin-depolymerizing activity comparable to that of the full-length side-chain analog (**2**) of aplyronine A.

Acknowledgments

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12. *Satisfactory spectroscopic data were obtained for synthetic analogs*. Compound **3**: TLC, R_f 0.38 (benzene–EtOAc–MeOH 3:3:1); $[\alpha]_D^{25}$ −37.5 (*c* 0.20, $CHCl_3$); IR (neat) 1738, 1696, 1657, 1451, 1374, 1347, 1319, 1236, 1171, 1076, 968, 727 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.28 [8.07] (s, 1H), 6.48 [7.15] (d,

J = 13.8 Hz, 1H), 5.66 (m, 1H), 5.43 (m, 1H), 5.34 (br d, J = 6.5 Hz, 1H), 4.99 (m, 1H), 4.83 (dd, J = 3.0, 10.0 Hz, 1H), 3.24 (q, J = 7.0 Hz, 1H), 3.03 [3.07] (s, 3H), 2.56 (m, 1H), 2.37 (s, 6H), 2.09 (s, 3H), 1.81 (m, 1H), 1.70 (d, J = 6.2 Hz, 3H), 1.31 (d, J = 7.0 Hz, 3H), 1.02 (d, J = 7.0 Hz, 3H), 0.96 (d, J = 7.0 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets; ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 172.2, 170.3, 161.9, 129.2, 128.9, 127.9, 110.4, 77.1, 72.8, 62.9, 41.6, 39.4, 36.8, 33.0, 27.6, 20.9, 19.3, 17.8, 15.4, 10.4. Compound **4**: TLC, R_f 0.44 (benzene–EtOAc–MeOH 3:3:1); $[\alpha]_D^{25}$ −40.0 (c 0.10, $CHCl_3$); IR (neat) 1738, 1693, 1656, 1450, 1373, 1235, 1168, 1077, 968, 751, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 [8.07] (s, 1H), 7.36–7.23 (m, 5H), 6.55 (d, J = 16.0 Hz, 1H), 6.49 [7.17] (d, J = 14.4 Hz, 1H), 6.13 (m, 1H), 5.58 (m, 1H), 5.01 (m, 1H), 4.89 (dd, J = 2.4, 10.0 Hz, 1H), 3.29 (q, J = 6.8 Hz, 1H), 3.03 [3.07] (s, 3H), 2.57 (m, 1H), 2.37 (s, 6H), 2.11 (s, 3H), 1.95 (m, 1H), 1.36 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets. Compound **5**: TLC, R_f 0.47 (benzene–EtOAc–MeOH 3:3:1); $[\alpha]_D^{25}$ −24.0 (c 0.10, $CHCl_3$); IR (neat) 1738, 1693, 1657, 1453, 1374, 1235, 1170, 1076, 969, 748, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 [8.08] (s, 1H), 7.28–7.13 (m, 5H), 6.48 (d, J = 14.0 Hz, 1H), 5.64 (m, 1H), 5.40 (m, 1H), 5.35 (br d, J = 5.1 Hz, 1H), 4.97 (m, 1H), 4.81 (dd, J = 2.8, 9.9 Hz, 1H), 3.24 (q, J = 7.0 Hz, 1H), 3.04 [3.08] (s, 3H), 2.68 (t, J = 7.7 Hz, 2H), 2.52 (m, 1H), 2.37 (s, 6H), 2.09 (s, 3H), 1.78 (m, 1H), 1.29 (d, J = 7.0 Hz, 3H), 1.03 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets. The minor counterpart of the signal of 6.48 ppm was overlapped with those of 7.28–7.13 ppm. Compound **6**: TLC, R_f 0.50 (benzene–EtOAc–MeOH 3:3:1); $[\alpha]_D^{25}$ −31.0 (c 0.50, $CHCl_3$); IR (neat) 1738, 1694, 1656, 1453, 1373, 1235, 1170, 1076, 968, 749, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 [8.08] (s, 1H), 7.27–7.14 (m, 5H), 6.48 (d, J = 14.0 Hz, 1H), 5.65 (m, 1H), 5.37 (m, 1H), 5.37 (m, 1H), 4.98 (m, 1H), 4.83 (dd, J = 2.8, 9.9 Hz, 1H), 3.23 (q, J = 7.0 Hz, 1H), 3.03 [3.07] (s, 3H), 2.59 (t, J = 7.6 Hz, 2H), 2.59 (m, 1H), 2.37 (s, 6H), 2.09 (s, 3H), 1.81 (m, 1H), 1.60 (m, 2H), 1.40 (m, 2H), 1.30 (d, J = 6.8 Hz, 3H), 1.03 (d, J = 6.4 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets. The minor counterpart of the signal of 6.48 ppm was overlapped with those of 7.27–7.14 ppm.

13. IC_{50} values of aplyronine A in the presence (5 mg/mL) and absence of analog **6** were 0.2 ng/mL and 0.2 ng/mL, respectively.