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Actin depolymerizing effect of trisoxazole-containing macrolides

Soon-Chun Chung [a], So-Hyoung Lee [a], Kyoung Hwa Jang [b], Wanki Park [a], Ju-eun Jeon [b], Hana Oh [b], Jongheon Shin [b,*], Ki-Bong Oh [a,*]

[a] Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea
[b] Natural Products Research Institute, College of Pharmacy, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea

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ABSTRACT

Oxazole-containing macrolides (**1**–**5**) isolated from the marine sponge *Chondrosia corticata* were evaluated for their actin depolymerizing activities by monitoring fluorescent intensity of pyrene F-actin. These studies led to the identification of (19*Z*)-halichondramide (**5**) as a new actin depolymerizing agent. The actin depolymerizing activity by (19*Z*)-halichondramide (**5**) was four times more potent than that of halichondramide (**1**). Compounds **1** and **5** also have potent antifungal activity. The preliminary structure–activity relationship of these compounds is described to elucidate the essential structural requirements.

Actin is one of the most abundant and common cytoskeletal proteins for cell growth, motility, signaling and maintenance of cell shape.[1] The dynamic interchange between monomeric G-actin and polymeric F-actin filaments is fundamental to cell motility and cytokinesis. Given the importance of the actin cytoskeleton in cellular function, it is not surprising that many naturally occurring compounds interfere with normal actin filament dynamics and regulation. One class of these is the extended family of marine trisoxazole-containing macrolides that includes kabiramides, jaspisamides, halichondramides, mycalolides, ulapualides, and halishigamides,[2] which are synthesized by marine sponges and exert their cytotoxic effects on cells by interfering with the regulation of actin filament dynamics.[3] Functionally, these compounds target actin with high affinity and specificity, exhibiting potent filament servering and monomer sequestering activities that ultimately result in cell death.

Trisoxazole-containing macrolides and structurally related marine metabolites are widely recognized to exhibit potent and diverse bioactivities such as antifungal activity, cytototoxicity, and icthyotoxicity as well as inhibition of cell division in fertilized sea urchin eggs.[4] The structural uniqueness and potent bioactivity of these compounds have attracted considerable biomedical and synthetic interest. Since the concurrent reports of ulapualides and kabiramide C from nudibranch egg masses in the late 1980s,[5] compounds of this structural class have been isolated from the nudibranch *Hexabranchus sanguineus*[6,7] and its potential prey, sponges of the genera *Halichondria*,[6,8] *Jaspis*,[9] and *Mycale*,[10] and even a taxonomically unrelated stony coral of the genus *Tubastrea*.[11]

In a previous work,[12] we had collected the sponge *Chondrosia corticata* Thiel (order Chondrosida, family Chondrillidae), from reef slopes on the south side of Cocos Lagoon, Guam. The crude extract of these specimens exhibited significant cytotoxicity against the human leukemia cell-line K562 as well as antifungal activity against *Candida albicans*. Bioassay-guided separation of the crude extracts using various chromatographic techniques yielded four known compounds including halichondramide (**1**), jaspisamide A (**2**), and halishigamide D (**3**) along with three new oxazole metabolites including neohalichondramide (**4**) and (19*Z*)-halichondramide (**5**) (Fig. 1). Herein, we describe the bioactivity of these compounds toward actin depolymerization and antimicrobial activity. These studies led to the identification of (19*Z*)-halichondramide (**5**) as a potent actin depolymerizing agent. The preliminary structure–activity relationship of these compounds is described to elucidate the essential structural requirements.

Oxazole-containing macrolides (**1**–**5**) were isolated from the marine sponge *C. corticata* by the method described previously.[12] The effect of compounds **1**–**5** on actin depolymerization was evaluated according to the methods outlined in the manufacture's protocol.[13] In this assay, pyrene G-actin had been assembled into fully fluorescent filamentous F-actin, and the progress of depolymerization was monitored by the decrease of fluorescence. As shown in Figure 2, the preassembled pyrene F-actin was dose-dependently depolymerized by the trisoxazole-containing macrolide **1** and **5**. When compound **1** (7.28 μM) and **5** (1.82 μM) were added to the pyrene F-actin, the fluorescence decreased immediately to the

* Corresponding authors. Tel.: +82 2 880 2484; fax: +82 2 762 8322 (J.S.); tel.: +82 2 880 4646; fax: +82 2 873 3112 (K.-B.O.).
E-mail addresses: shinj@snu.ac.kr (J. Shin), ohkibong@snu.ac.kr (K.-B. Oh).

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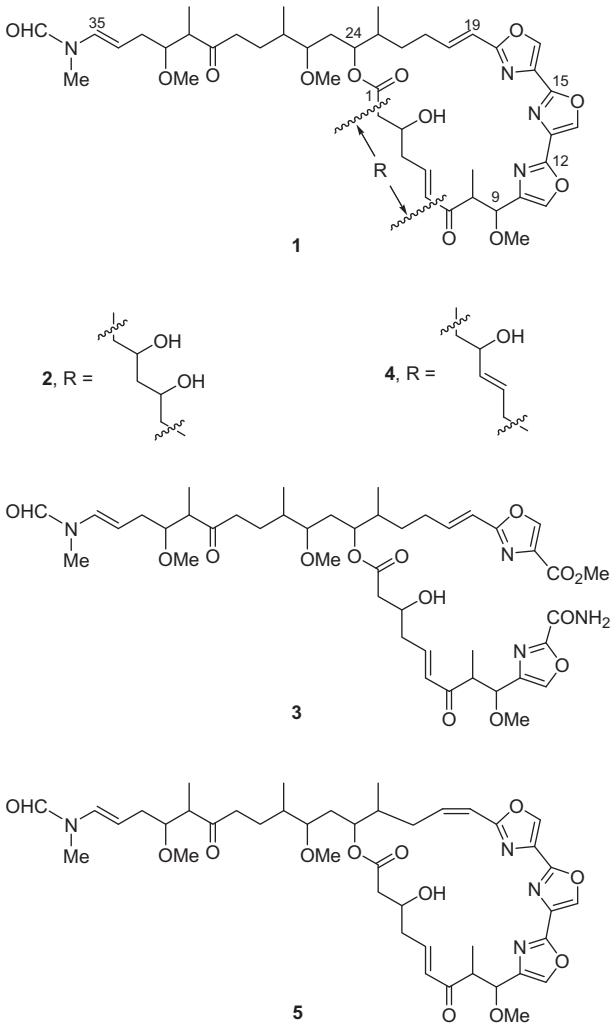

Figure 1. Chemical structure of marine trisoxazole-containing macrolides: halichondramide (**1**); jaspisamide A (**2**); halishigamide D (**3**); neohalichondramide (**4**); (19*Z*)-halichondramide (**5**).

basal level, indicating that the actin filaments were quickly depolymerized (>90%). The actin depolymerizing activity by compound **5** was **4** times more potent than that of compound **1**. In contrast, compounds **2** and **4** showed minor effects on the level of actin depolymerization compared with negative controls (F-actin alone) at the concentrations in the range of 14–114 μM and 15–120 μM, respectively (Fig. 2). Compound **3** also showed weak actin depolymerizing activity (40% depolymerization at 112 μM) (data not shown).

The X-ray structure of actin in complex with jaspisamide A (**2**) and a simple model to explain trisoxazole macrolide-mediated severing have been reported elsewhere.[3] Jaspisamide A (**2**) binds in the cleft between F-actin subdomains **1** and **3**, with the macrolide ring interacting primarily with a hydrophobic patch formed by actin residues Ile341, Ile345, Ser348 and Leu349. This interaction is weak but serves to anchor the macrolide to actin. Thereafter, the long, hydrophobic and flexible tail of the toxin inserts in the cleft between subdomains **1** and **3**.[3a] As shown in Fig. 1, compounds **1**, **2** and **4** are almost identical in structure and conformation, except for the substituents at C-5 and C-6 (Fig. 1). In compound **1**, C-5 and C-6 are connected by double bond, whereas in compounds **2** and **4** this bond is hydrated and migrated to C-4, respectively. Interestingly, the actin depolymerizing activity of compounds **2** and **4** was significantly diminished compared with compound **1** as shown in Fig. 2. These results are supposedly due to decreased hydrophobicity of macrolide ring to anchor the macrolides **2** and **4** to actin. Comparison of the chemical structure of compound **1** and compound **5** suggests that the macrolide ring but not the side-chain of compound **5** may account for its severing activity. Compound **5** possessed the 19*Z* double bond instead of the 19*E* geometry found in **1** (Fig. 1).[12] A three-dimensional model study using structurally related compounds revealed that the 19*E* (**1**) double bond placed the H-20 olefinic proton spatially proximal to the oxazole ring, while the 19*Z* (**5**) geometry reversed the orientation and located the H-21 allylic protons near to the oxazole ring. Compound **3** was structurally defined to be a derivative of compound **1**, in which the C-13–C-15 oxazole ring was cleaved to a methyl ester and primary amide. The actin depolymerizing activity of compound **3** was also diminished compared with entire trisoxazole ring compound **1**. Taken together, our data suggest that C-5–C-6 double bond and 19*Z* double bond geometry are the key determinants of actin depolymerizing activity in trisoxazole-containing macrolides.

The in vitro antimicrobial activity of the compounds **1**–**5** was assessed against three representative Gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538p), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (IFO 12708), three Gram-negative bacteria, *Proteus vulgaris* (ATCC 3851), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 25922), and four fungi, *C. albicans* (ATCC 10231), *Aspergillus fumigatus*

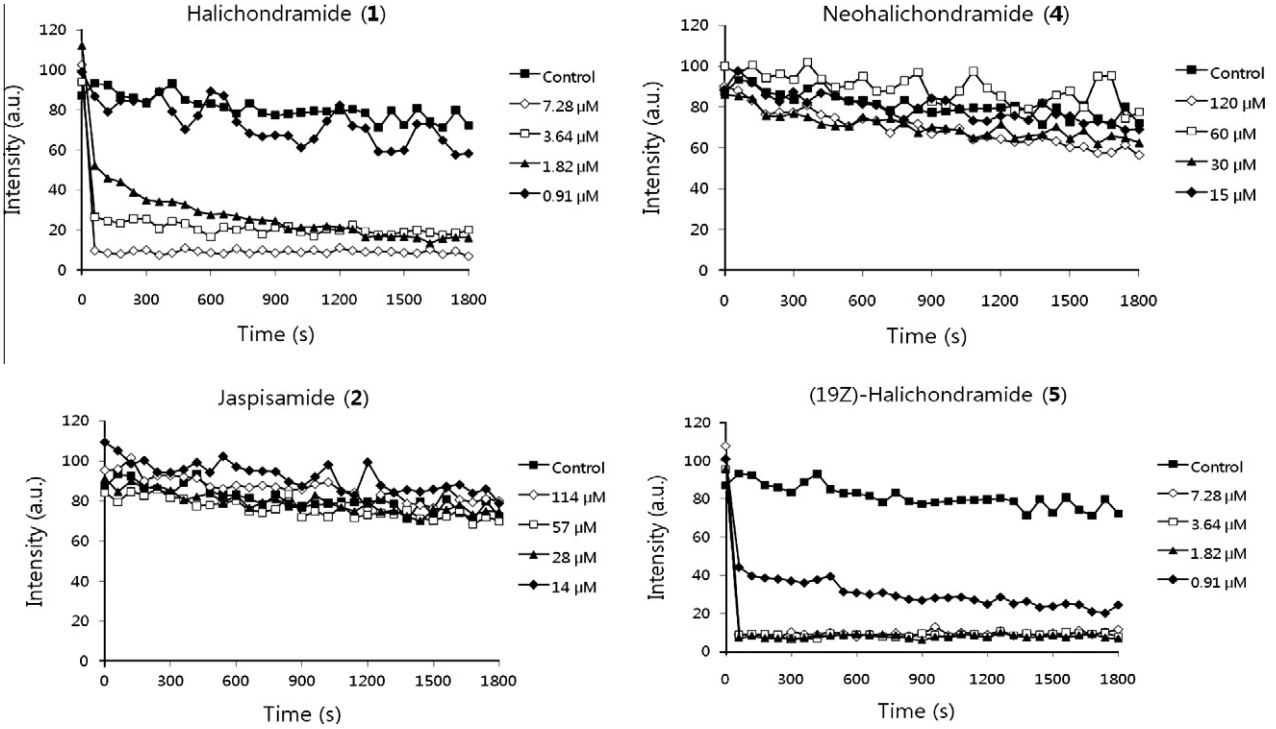

Figure 2. Effects of halichondramide (**1**), jaspisamide A (**2**), neohalichondramide (**4**), and (19*Z*)-halichondramide (**5**) on F-actin depolymerization assayed by fluorometry. The progress of pyrene F-actin depolymerization was measured as arbitrary fluorescence intensity (Intensity a.u.). Pyrene F-actin alone (4.8 μM) served as negative controls.

Table 1
MIC of compounds **1–5** against fungal strains[a,b]

Compound	MIC (μg/ml, μM)			
	Ca	Af	Tr	Tm
1	0.20 (0.23)	0.39 (0.45)	0.39 (0.48)	0.78 (0.91)
2	>100	>100	>100	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
5	0.78 (0.91)	12.5 (14.55)	6.25 (7.27)	12.5 (14.55)
Amphotericin B	0.20 (0.22)	0.78 (0.84)	0.78 (0.84)	0.78 (0.84)

[a] MIC value represents concentration giving complete inhibition relative to the negative control.
[b] Microorganisms: Ca, *Candida albicans* ATCC 10231; Af, *Aspergillus fumigatus* HCI 6094; Tr, *Trichophyton rubrum* IFO 9185; Tm, *Trichophyton mentagrophytes* IFO 40996.

(HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996).[14,15] In an antibacterial activity assay, all of these compounds **1–5** did not inhibit a range of Gram-positive or Gram-negative bacteria at 100 μg/ml (data not shown). The MICs of the tested compounds against fungal strains are displayed in Table 1. Compounds **1** and **5** both showed potent inhibitory activity against *C. albicans* with MIC values of 0.23 and 0.91 μM, respectively, as shown in comparison to amphotericin B. In this measurement, compounds **2–4** were inactive at 100 μg/ml. A similar trend was also observed in the antifungal activity test against the fungi *A. fumigatus*, *T. rubrum* and *T. mentagrophytes*. The trends observed in antifungal activity data roughly parallel those of actin depolymerizing activity as described above. Oxazole-containing macrolides exhibit potent cytotoxicity and antifungal activity that is attributable to the actin depolymerizing ability of these metabolites.[16]

In conclusion, five oxazole-containing macrolides were isolated from the marine sponge *C. corticata* and their activities toward actin depolymerization were investigated. These studies led to the identification of compound 5 as a new and promising lead compound for the development of potent actin depolymerizing agents. Our data suggest that C-5–C-6 double bond and 19*Z* double bond geometry are the key determinants of actin depolymerizing activity in trisoxazole-containing macrolides. Compounds **1** and **5** also have potent antifungal activity that is attributable to the actin depolymerizing ability of these metabolites.

Acknowledgments

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13. Actin depolymerization assay: the rate of actin depolymerization in the presence of fluorescent filamentous F-actin was monitored according to the methods outlined in the Actin Polymerization Biochem Kit from Cytoskeleton Inc.. The pyrene G-actin was prepared to 24 μM by adding G-buffer. Subsequently, 10× actin polymerization buffer was added to final concentration at 0.25×, and incubated at room temperature for 1 h. This polymerized pyrene F-actin solution was diluted with G-buffer to prepare 5-fold dilutions. 100 μl pyrene F-actin solution (final concentration of pyrene F-actin, 4.8 μM) was used to actin depolymerization assay. Measurement of the fluorescence was performed in a spectrophotometer (Perkin–Elmer) with excitation/emission wavelength at 360 and 410 nm, respectively, for 1 h every 1 min.
14. Antibacterial activity assay: bacteria were grown overnight in Luria–Bertani (LB) broth at 37 °C, harvested by centrifugation, and then washed twice with sterile distilled water. Each test compound was dissolved in DMSO and diluted with Standard methods broth (Difco) to prepare serial twofold dilutions in the range of 100–0.05 μg/ml. Ten microliters of the broth containing approximately 10^5 colony-forming units (cfu)/ml of test bacteria was added to each well of a 96-well microtiter plate. Culture plates were incubated for 24 h at 37 °C. The minimum inhibitory concentration (MIC) values were determined as the lowest concentration of test compounds that inhibited bacterial growth. Ampicillin was used as a reference compound.
15. *Antifungal activity assay*: *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone and 2% dextrose), harvested by centrifugation, and then twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (Difco) and incubated for 2 weeks at 28 °C. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial 2-fold dilutions in the range of 100–0.05 μg/ml. Ten microliters of the broth containing approximately 10^4 cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for 48–72 h at 28 °C. The MIC values were determined as the lowest concentration of test compounds that inhibited fungal growth. Amphotericin B was used as a reference compound.
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