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Seragamides A–F, new actin-targeting depsipeptides from the sponge *Suberites japonicus* Thiele

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Abstract—Six new depsipeptides, seragamides A–F (**1**–**6**), and a known geodiamolide I (**7**) have been isolated as cytotoxic metabolites from the Okinawan sponge *Suberites japonicus*. Their structures were elucidated by means of spectroscopic analysis and chemical transformations. Seragamide A (**1**) promotes the polymerization of G-actin and stabilizes F-actin filaments.

1. Introduction

The eukaryotic cytoskeleton is composed of three groups of protein polymers: microtubules, intermediate filaments and microfilaments. Among these, the microtubule apparatus is critical for mitosis and is the target of anticancer agents that include vinca alkaloids and paclitaxel. The microfilament apparatus on the other hand is rarely considered as a target for chemical therapy. Recent studies, however, suggest that actin and the actin filament system of tumor cells, like microtubules, may serve as a critical target for small molecule drugs.[1]

Microfilaments are composed of F-actin and associated proteins; these filamentous assemblies play essential roles in cytokinesis, cell motility, and cell adhesion.[2] Actin filament dynamics in eukaryotic cells is regulated by a large number of actin-binding proteins (ABP) such as cofilin, gelsolin, thymosin β4 and profilin. Given the complexity of the cell cytoskeleton, studies that aim to understand the regulation of actin filament dynamics usually employ small molecule drugs such as fluorescent phalloidin, a marker of F-actin, and cytochalasin D, which inhibits actin polymerization. Recently, several highly specific actin-targeting drugs have been identified from marine natural products. The first, and most frequently used marine-derived drugs are latrunculins A and B, which block polymerization by plugging the ATP site of G-actin.[3] Other actin-targeting marine drugs include jaspamide/jasplakinolide, which stabilizes F-actin,[4,5] trisoxazole macrolides,[6–8] swinholide A,[9] hectochlorin,[10] and amphidinolide H.[11]

During a functional screen for cytotoxic drugs that inhibit the actin-driven processes of cytokinesis, we found that a lipophilic extract of the yellow sponge *Suberites japonicus* killed cells at 2 μg/mL with the formation of characteristic morphological changes similar to that found for trisoxazole macrolides, swinholide A, and misakinolide A. At a lower concentration range of 0.2–0.02 μg/mL, the extract led to the formation of multiple nuclei, which suggests that the active drug might target the actin cytoskeleton. We isolated and chemically characterized the bioactive products and determined their mode of action. The seragamides A–F identified from these studies represent new and novel molecular tools to study the regulation of the actin cytoskeleton and may prove useful as anti-cancer drugs.

2. Results and discussion

2.1. Structures of 1–7

A small amount of the sponge *S. japonicus* Thiele was initially collected at Seragaki, Okinawa. Its lipophilic extract gave rise to two new cytotoxic depsipeptides named seragamides A (**1**) and B (**2**). A larger amount of the sponge collected off Manza yielded seragamides C–F (**3**–**6**) in addition to **1**, **2**, and the known geodiamolide I (**7**).

Keywords: Geodiamolide; Actin polymerization; Cytokinesis; Jaspamide; Jasplakinolide.
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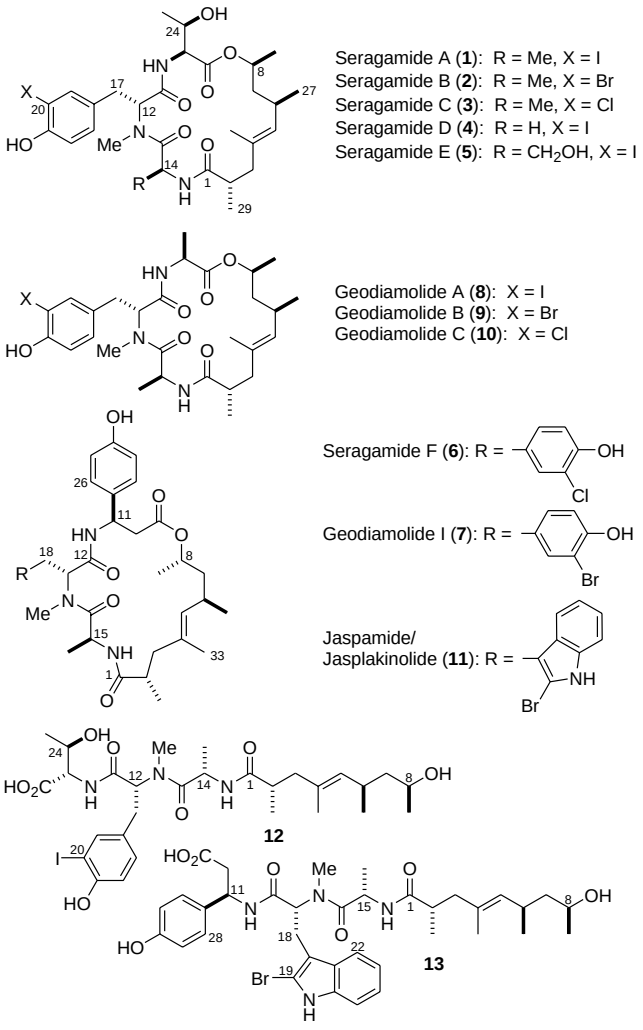

Seragamide A (**1**) obtained as an amorphous solid was analyzed for $C_{29}H_{42}N_3O_7I$ by HRFABMS. It showed IR absorption bands due to hydroxyl (3320 cm^{-1}), ester (1732 cm^{-1}), and amide (1635 cm^{-1}) functionalities. The peptidic nature of compound **1** was suggested by two amide signals (δ 6.49 and 6.71; δ 170–176) in NMR in addition to the IR absorption. The ^{1}H NMR data exhibited the presence of an *N*-methyl (δ 3.00) and aromatic protons (δ 6.90, 7.09, and 7.50). NMR analysis indicated the presence of three amino acid residues: Thr, iodo-*N*-methyl-tyrosine (I-*N*-Me-Tyr), and Ala.

A downfield signal (δ 6.71) for an amide proton showed a COSY correlation with a methine proton signal at δ 4.42 (δ 58.1), which in turn coupled to an oxymethine proton resonance at δ 4.29 (δ 67.7). This signal further coupled to a methyl signal at δ 1.09 (δ 18.7). Thus, this unit was deduced to Thr. The amide proton signal showed HMBC correlation to a carbonyl carbon signal (δ 170.0), while a resonance (δ 4.42) for an alpha proton showed cross peaks with δ 170.0 and another carbonyl at δ 169.4.

Second amino acid residue was assigned as I-*N*-Me-Tyr as follows. An *N*-methyl resonance (δ 3.00) showed an HMBC cross peak to an alpha carbon signal at δ 56.7. A signal (δ 5.32) for the alpha proton showed COSY cross peaks with downfield shifted β-methylene signals (δ 2.93 and 3.20). The presence of a 1,2,4-trisubstituted benzene ring was shown by observing three aromatic protons at δ 6.90 (d, J= 8.5 Hz), 7.09 (dd, J=8.5, 2.1 Hz), and δ 7.50 (d, J= 2.1 Hz). The chemical shifts of the proton at δ 6.90 and the carbon at δ 154.0 suggested the ring to be phenolic. The β-methylene protons showed HMBC correlation with three aromatic carbons at δ 130.3, 138.2 and 130.6, whereas aromatic protons at δ 7.50 and 7.09 showed correlation to the beta carbon at δ 32.8 indicating the residue as a tyrosine derivative. The aromatic signal (δ 7.50) showed correlation to a characteristic high-field carbon signal at δ 85.5, suggesting the presence of iodine atom on this carbon. The ^{1}H and ^{13}C NMR data for the iodo-tyrosine residue were comparable with those of the same moiety of geodiamolide A (**8**).[12]

The last amino acid unit was assigned as Ala by observing ^{1}H NMR signals for a methine at δ 4.77, a methyl at δ 1.13, and an amide proton at δ 6.49. Both the methyl and the methine protons showed HMBC correlation to a carbonyl carbon at δ 174.5, while the amide proton exhibited HMBC cross peak to another carbonyl at δ 175.3.

The remaining portion of the molecule $C_{12}H_{20}O_2$ was elucidated as a polyketide composed of four units of propionates by the following NMR data. Four methyl groups were separated into three aliphatic doublets (δ 0.87, 1.15, 1.25) and one vinyl methyl (δ 1.52). HMBC correlations from these four methyl groups to the rest of the residue allowed us to construct a fatty acid residue as 2,4,6-trimethyl-non-4-enoyl, the same moiety observed in geodiamolides and jaspamides. The *E* configuration of the 4,5-double bond was determined by the NOE signals between the vinyl methyl (δ 1.52) and the methine proton (δ 2.22) at C-6, and between the olefinic proton (δ 4.91br d) and one of the methylene protons (δ 2.02) at C-3.

The cyclic nature of seragamide A (**1**) was evident from its ten degrees of unsaturation, nine of which were accounted for four carbonyls, one aromatic, and one double bond. Connection of the amino acid residues and the alkanoate unit was obtained by HMBC correlations: the amide proton at δ 6.71 and a carbonyl of I-*N*-Me-Tyr at δ 170.0s, *N*-methyl group of I-*N*-Me-Tyr at δ 3.00 and a carbonyl of Ala at δ 174.5s, the amide proton at δ 6.49 and a carbonyl of the alkanoate at δ 175.3s, and the oxymethine proton at δ 4.87 and the ester carbonyl at δ 169.4s. Therefore, the structure of seragamide A was assembled as shown in **1**.

Absolute configurations of the amino acid residues were determined by applying Marfey's method on its acid hydrolysate (see Section 3). The result revealed the amino acids to be L-Ala, L-Thr, and D-*N*-Me-Tyr. All of the three amide bonds are in trans geometry as shown by NOEs between NH-Ala (δ 6.49) and H-2 (δ 2.36), NH-Thr (δ 6.71) and alpha proton (δ 5.32) of I-*N*-Me-Tyr, and *N*-Me-Tyr (δ 3.00) and alpha proton (δ 4.77) of Ala. When ^{1}H and ^{13}C NMR data of **1** were compared with those of **8** as shown in Table 1, most of the coupling constants and chemical shifts for the fatty acid portion were nearly identical suggesting that the three chiral centers were the same as those of geodiamolide A (**8**) and its congeners. For further

Table 1. NMR data for seragamide A (**1**) and for geodiamolide A (**8**)

	Seragamide A (**1**)				**8**[12]	
C#	^{13}C	^{1}H (*J* in Hz)	HMBC	COSY	^{13}C	^{1}H
1	175.3s				170.9s	
2	42.0d	2.36ddq (4.0, 11.0, 7.0)		H-3ab,29	42.4d	2.32m
3	42.9t	a2.02dd (4.0, 14.0)	C-4	H-2,3b,5	43.3t	a2.04dd
		b2.16m	C-4	H-2,3a		b2.16d
4	133.2s				129.6s	
5	131.2d	4.91br d (9.0)	C-27	H-3a,6,28	131.6d	4.93d
6	29.0d	2.22m		H-5,7ab,27	29.0d	2.16m
7	43.4t	a1.38ddd (5.8, 8.0, 13.7)	C-5,6,26,27	H-6,7b,8	43.7t	a1.34m
		b1.63m	C-5,6,26,27	H-6,7a,8		b1.59m
8	71.8d	4.87ddq (6.0, 7.0, 6.5)	C-7,9	H-7ab,26	71.0d	4.91m
9	169.4s				168.8s	
10	58.1d	4.42dd (3.0, 8.5)	C-9,11	H-10NH	49.0d	4.75dq
11	170.0s				175.1s	
12	56.7d	5.32dd (7.6, 9.2)	C-11,13,16,17	H-17ab	56.7d	5.21dd
13	174.5s				174.4s	
14	45.8d	4.77dq (6.7, 6.7)	C-13,15	H-14NH,15	45.9d	4.46dq
15	19.7q	1.13d (6.7)	C-13,14	H-14	18.8q	1.34q
16	30.6q	3.00s	C-12,13		30.7q	2.97s
17	32.8t	a2.93dd (9.2, 14.6)	C-11,12,19,23	H-12,17b	32.7t	a2.95dd
		b3.20dd (7.6, 14.6)	C-12,18,19	H-12,17a		b3.15dd
18	130.3s				133.0s	
19	138.2d	7.50d (2.1)	C-17,18,20,21	H-23	132.2d	7.29d
20	85.5s				85.1s	
21	154.0s				154.5s	
22	115.1d	6.90d (8.5)	C-18,20,21,23	H-23	116.1d	6.87d
23	130.6d	7.09dd (2.1, 8.5)	C-17,19,21	H-19,22	129.4d	7.05dd
24	67.7d	4.29dq (3.0, 6.5)		H-25	18.7q	1.02d
25	18.7q	1.09d (6.5)	C-10,24	H-24		
26	20.6q	1.25d (6.5)	C-7,8	H-8	20.6q	1.24d
27	20.5q	0.87d (6.5)	C-5,6,7	H-6	18.2q	0.86d
28	17.8q	1.52d (1.2)	C-3,4,5	H-5	17.7q	1.49s
29	18.9q	1.15d (7.0)	C-1,3	H-2	20.4q	1.14d
10NH		6.71d (8.5)	C-11	H-10		6.52d
14NH		6.49d (6.7)	C-1	H-14		6.59d
TyrOH		5.55br s				6.27s
ThrOH		3.49s				

confirmation, ring-opened derivatives **12** and **13** were prepared by saponification of **1** and **11**, respectively, and their NMR data were compared. Except for the H-2 chemical shifts ($\Delta\delta$ 0.09), all other chemical shifts and coupling constants for the polyketide portion were in good agreement as shown in Table 2, indicating the same stereochemistry for the portion in **1** and **11**. Thus, we concluded the stereochemistry of seragamide A (**1**) as shown.

The molecular formula of seragamide B (**2**), $C_{29}H_{42}N_3O_7Br$, suggested that it had a bromine atom instead of an iodine atom in **1**. The IR spectrum showed similar absorptions (3320, 1733, 1650 cm^{-1}) to those of **1**. Inspection of NMR spectra of **2** indicated that almost all signals in **2** are the same with those of **1** except for the aromatic portion of Tyr residue (Tables 1 and 3). Further comparison of the data with those of geodiamolide B (**9**),[12] which retains Br-*N*-Me-Tyr residue, confirmed that **2** contained a bromine atom in the place of iodine in **1**. Marfey analysis of the acid hydrolysate of **2** determined the residues as L-Ala and L-Thr. As in the discussion of seragamide A (**1**), the remaining chiralities of **2** are regarded to be the same.

Table 2. Comparison of the ^{1}H NMR data for the polyketide portion of **12** and **13** (*J* in Hz)

H#	12	13
2	2.47ddq (7.1, 8.1, 6.8)	2.38ddq (7.3, 8.0, 6.8)
2-Me	1.04d (6.8)	1.02d (6.8)
3a	1.96dd (8.1, 13.9)	1.94dd (8.0, 13.7)
3b	2.26dd (7.1, 13.9)	2.25dd (7.3, 13.7)
4-Me	1.57s	1.56s
5	4.95d (9.3)	4.91d (9.8)
6	2.43dddq (5.9, 8.8, 9.3, 6.6)	2.39dddq (5.9, 9.8, 9.3, 6.6)
6-Me	0.89d (6.6)	0.87d (6.6)
7a	1.34ddd (5.9, 13.4, 6.1)	1.31ddd (5.9, 13.4, 6.1)
7b	1.43ddd (8.8, 13.4, 6.1)	1.41ddd (9.3, 13.4, 6.1)
8	3.69ddq (6.1, 6.1, 6.1)	3.68ddq (6.1, 6.1, 6.1)
8-Me	1.12d (6.1)	1.12d (6.1)

Seragamide C (**3**) was found to have a molecular formula $C_{29}H_{42}N_3O_7Cl$ by HRFABMS. Except for aromatic portions of Tyr residue in **3**, most NMR signals are similar to those of **1** and **2**. The aromatic protons (δ 6.94, 7.03, 7.17) are virtually identical to those (δ 6.93, 7.01, 7.15) of geodiamolide C (**10**),[13] which has Cl-*N*-Me-Tyr residue. The structure of **3** was elucidated as shown.

The molecular formula of seragamide D (**4**), $C_{28}H_{40}N_3O_7I$, suggested that it is a demethyl analogue of seragamide A (**1**). The methyl group (δ 19.7) of Ala residue in **1** disappeared in **4**, and two characteristic downfield shifted methylene protons (δ 3.80, 4.17) appeared instead. As most other portions showed similar NMR data (Tables 3 and 4) as **1**, seragamide D (**4**) was elucidated as a congener having Gly in the place of Ala in seragamide A (**1**). The linkage of

Table 3. ^{1}H NMR data for seragamides B–E (**2**–**5**) (*J* in Hz)

	B (**2**)	C (**3**)	D (**4**)	E (**5**)
2	2.36m	2.36m	2.47tq (7.9, 6.7)	2.42ddq (4.0, 11.0, 6.7)
3a	2.02dd (3.0, 14.0)	2.02dd (3.0, 14.0)	2.12d (7.9, 2H)	2.08dd (4.0, 14.0)
3b	2.17dd (11.5, 14.0)	2.17dd (11.3, 14.0)		2.16m
5	4.91d (10.0)	4.91d (8.8)	4.98d (7.9)	4.93d (9.2)
6	2.22m	2.20m	2.23m	2.20m
7a	1.39m	1.39ddd (6.1, 7.9, 13.7)	1.44ddd (6.8, 8.2, 13.7)	1.39ddd (5.2, 7.9, 13.7)
7b	1.64m	1.63ddd (6.7, 7.1, 13.7)	1.70ddd (6.1, 6.1, 13.7)	1.60m
8	4.87ddq (5.2, 7.2, 6.5)	4.87ddq (6.1, 7.1, 6.4)	4.80ddq (6.1, 6.8, 6.1)	4.92m
10	4.42dd (3.1, 8.8)	4.42dd (3.0, 8.8)	4.46dd (2.4, 9.2)	4.41dd (2.7, 8.8)
12	5.33dd (7.3, 9.2)	5.34dd (7.6, 8.8)	5.18dd (6.4, 9.5)	5.31dd (7.6, 8.8)
14	4.77dq (6.5, 6.5)	4.77dq (6.7, 6.7)	a3.80dd (3.0, 17.7) b4.17dd (4.3, 17.7)	4.83m
15	1.12d (6.5)	1.12d (6.7)		3.59m (2H)
16	3.00s	3.00s	2.96s	3.08s
17a	2.94dd (9.2, 14.6)	2.93dd (8.8, 14.6)	2.83dd (6.4, 13.7)	2.93dd (8.8, 14.6)
17b	3.21dd (7.3, 14.6)	3.21dd (7.6, 14.6)	3.26dd (9.5, 13.7)	3.23dd (7.6, 14.6)
19	7.31d (2.1)	7.17d (2.1)	7.53d (1.8)	7.50d (2.1)
22	6.94d (8.3)	6.94d (8.2)	6.91d (8.5)	6.91d (8.5)
23	7.07dd (2.1, 8.3)	7.03dd (2.1, 8.2)	7.10dd (1.8, 8.5)	7.09dd (2.1, 8.5)
24	4.29m	4.30m	4.22m	4.33m
25	1.09d (6.5)	1.09d (6.4)	0.99d (6.7)	1.08d (6.4)
26	1.25d (6.5)	1.25d (6.4)	1.27d (6.1)	1.25d (6.1)
27	0.88d (6.5)	0.87d (6.7)	0.90d (6.7)	0.89d (6.7)
28	1.52d (1.2)	1.52d (1.2)	1.56s	1.52d (1.2)
29	1.15d (7.0)	1.15d (7.0)	1.17d (6.7)	1.18d (6.7)
10NH	6.72d (8.8)	6.69d (8.8)	6.64d (9.2)	6.66d (8.8)
14NH	6.48d (6.5)	6.47d (6.7)	6.48br s	6.70d (6.4)
15OH				3.55m
21OH	5.55br s	5.52br s	5.35s	5.47br s
24OH	3.49br s	3.49br s	3.49br s	3.50d (4.9)

the residues was confirmed by HMBC correlations (H-10/C-11, NH-Thr/C-11, *N*-Me/C-12,13, H-14/C-1).

Seragamide E (**5**), $C_{29}H_{42}N_3O_8I$, was analyzed to have an additional oxygen atom on the formula of seragamide A (**1**). In the NMR spectra of **5**, it lacked methyl signals corresponding to Ala residue in **1**, while it exhibited signals for a primary alcohol (δ 65.1; δ 3.59), suggesting the presence of Ser residue instead of Ala in **1**. This disposition was also supported by HMBC correlations (*N*-Me/C-12,13, H-14/C-13). The remaining portions showed nearly the same NMR data with other seragamides (Tables 3 and 4). The absolute stereochemistry of Ser was determined to be L by Marfey's method.

Table 4. ^{13}C NMR data for seragamides B–E (**2**–**5**)

	B (**2**)	C (**3**)	D (**4**)	E (**5**)
1	175.2s	175.2s	175.8s	177.4s
2	41.9d	41.9d	41.8d	42.0d
3	42.8t	42.8t	42.9t	42.9t
4	133.2s	133.3s	133.5s	132.9s
5	131.1d	131.0d	131.0d	131.6d
6	29.0d	29.1d	29.2d	29.1d
7	43.4t	43.5t	43.4t	43.8t
8	71.8d	71.8d	72.6d	71.5d
9	169.4s	169.4s	169.2s	169.5s
10	58.0d	58.0d	57.8d	58.0d
11	169.9s	170.0s	169.2s	169.8s
12	56.6d	56.7s	57.7d	57.0d
13	174.5s	174.5s	169.9s	171.8s
14	45.8d	45.8d	42.1t	52.5d
15	19.7q	18.6q		65.1t
16	30.6q	30.6q	29.8q	31.0s
17	32.9t	33.0q	32.5t	32.8t
18	129.8s	129.5s	130.4s	130.2s
19	132.0d	129.0d	138.6d	138.2d
20	110.1s	119.8s	85.7s	85.6s
21	151.2s	150.3s	154.0s	154.0s
22	116.2d	116.4d	115.2d	115.2d
23	129.6d	128.8d	130.8d	130.6d
24	67.7d	67.7d	68.1d	67.6d
25	18.6q	19.7q	19.5q	19.8q
26	20.5q	20.5q	20.6q	20.7q
27	20.5q	20.6q	20.6q	20.5q
28	17.8q	17.9q	18.2q	18.0q
29	18.9q	19.0q	18.4q	18.9q

Seragamide F (**6**), $C_{34}H_{44}N_3O_7Cl$, showed the presence of two aromatic rings as observed for geodiamolide I (**7**).[14] Of the two aromatic rings in **6**, a *p*-disubstitued one is virtually the same as in **7**, while 1,2,4-trisubstituted one (δ 7.13d, 6.90d, 6.99dd) is similar to the one found for seragamide C (**3**) (δ 7.17d, 6.94d, 7.03dd). Therefore, the structural difference between **6** and **7** lies at the halogen substitution on the latter aromatic ring. Close similarities of NMR data of **6** to **7** indicate that they have the same stereochemistry.

2.2. Biological activity

The IC_{50} values of seragamides A–E (**1**–**5**) were determined as 0.064, 0.12, 0.10, 0.18 and 0.58 μM, respectively. Seragamides A–E (**1**–**5**) caused multinuclei formation in cells at 0.01, 0.02, 0.01, 0.01, and 0.04 μg/mL, respectively. Seragamide F (**6**) was not tested.

Using a Prodan-actin polymerization assay[15] we found that seragamide A (**1**) in the range of 20–200 nM facilitated the polymerization of 1 μM G-actin, as shown in Figure 1. In the Prodan-actin depolymerization assay, 1 nM seragamide A (**1**) inhibited the depolymerization of F-actin at 100 nM (Fig. 2). Since these activities are similar to those observed for jaspamide/jasplakinolide (**11**), we suggest that

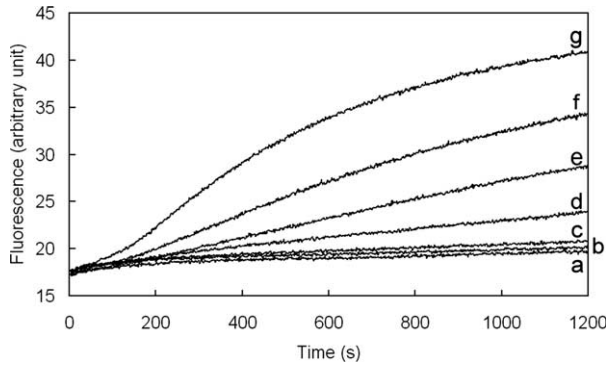

Figure 1. The effect of seragamide A (**1**) on polymerization of G-actin. Prodan-labeled G-actin (1 μM) is mixed with seragamide A (**1**) at the concentration of 0 nM (a), 1 nM (b), 10 nM (c), 20 nM (d), 50 nM (e), 100 nM (f), and 200 nM (g).

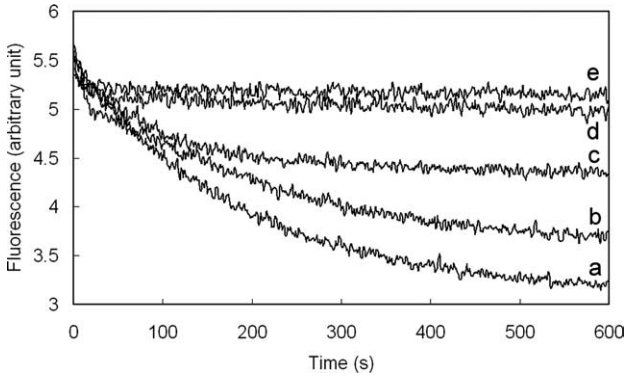

Figure 2. The effect of seragamide A (**1**) on depolymerization of F-actin. Prodan-labeled F-actin (28 μM) is mixed to a final concentration at 100 nM with seragamide A (**1**) at 0 nM (a), 0.05 nM (b), 0.1 nM (c), 1 nM (d), and 10 nM (e).

seragamides also function as cell permeable, F-actin stabilizing drugs.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Jeol α-500 spectrometer. Mass spectra were measured on Jeol JMS-D300. IR, UV spectra and the optical rotations were measured using a Shimadzu FTIR-8300A instrument, a Hitachi U-2001 spectrophotometer, and a Jasco DIP-1000 polarimeter, respectively. Fluorescence spectra were taken on a Jasco FP6500 fluorometer. A microplate reader TECAN GENios was used for cytotoxicity. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-6000 pump equipped with a Hitachi RI monitor (655A-30) and a Hitachi L-4000 UV detector, using Cosmosil packed ODS HPLC column (5C18-AR-II, 10× 250 mm). Merck silica gel was used for vacuum flash chromatography. Nacalai Cosmosil was used for reversed phase column chromatography. All solvents used were reagent grade.

3.2. Animal material

The yellow round sponge was identified as *S. japonicus* Thiele (Order Hadromerida, Family Suberitidae) by Dr. John N.A. Hooper, Queensland Museum, Brisbane. The specimen is deposited at the museum (QMG317382).

3.3. Extraction and isolation

The first collection (40 g, wet) of the sponge *S. japonicus* was made in 2001 by R.F.B. at a depth of 60 m by hand using SCUBA at Seragaki, Okinawa. The fresh sample was soaked and kept in MeOH until extraction. The MeOH solution was concentrated and the residual material was partitioned between EtOAc and H_2O. The residue of the EtOAc extract (33 mg) was chromatographed on a column packed with Cosmosil using MeOH–H_2O to give six fractions. These fractions were submitted for a cell-based high content imaging assay; fractions 1 and 2 were found to be active (fraction 1: 0.2 μg/mL and fraction 2: 2 μg/mL), showing vacuoles, blebs, arborization similar to that observed for misakinolide A.[16] Each fraction was separated by reversed phase HPLC using MeOH–H_2O (60/40) to give compounds **1** (4.1 mg) and **2** (1.6 mg).

The second specimen (0.5 kg, wet), collected at a depth of 50 m off Manza, was repeatedly extracted with MeOH and then with acetone. After concentration the combined extracts were partitioned between EtOAc and H_2O. The residue (6.4 g) of the organic extract was subjected to vacuum flash column chromatography (VFC) on silica gel (hexane, hexane–EtOAc, EtOAc, and EtOAc–MeOH) to afford four fractions. ^{1}H NMR spectrum of each fraction was compared with those of **1** and **2**. The spectrum of fraction **3** clearly indicated the presence of **1**. The fraction was roughly separated by MPLC (Lobar column) using 60% aqueous MeOH to give three subfractions. Each of these fractions was purified on reversed phase HPLC using the same solvent (60% aqueous MeOH) to afford compounds **1–7** in the amount of 145.8, 23.0, 2.0, 1.5, 1.8, 0.4, and 0.9 mg, respectively.

3.3.1. Seragamide A (1). White amorphous solid, $[\alpha]_D^{27}$ +45.6 (*c* 0.215, $CHCl_3$); IR (neat) ν_{max}: 3320, 1732, 1635 cm^{-1}; UV λ_{max} (MeOH): 207 (ε 3.4×10^4) and 286 nm (2.8×10^3); FABMS: *m/z* 672 ([M+H]$^+$), 546, 423; HRFABMS: *m/z* 672.2133, calcd for $C_{29}H_{43}N_3O_7I$ 672.2146; ^{1}H and ^{13}C NMR ($CDCl_3$): Table 1.

3.3.2. Seragamide B (2). White amorphous solid, $[\alpha]_D^{27}$ +39 (*c* 0.090, $CHCl_3$); IR (neat) ν_{max}: 3320, 1733, 1650 cm^{-1}; UV λ_{max} (MeOH): 205 (ε 3.1×10^4) and 283 nm (2.3×10^3); FABMS: *m/z* 626, 624 ([M+H]$^+$); HRFABMS: 624.2240, calcd for $C_{29}H_{43}N_3O_7{}^{79}Br$ 624.2284; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4.

3.3.3. Seragamide C (3). Colorless glass, $[\alpha]_D^{23}$ +53 (*c* 0.10, $CHCl_3$); IR (neat) ν_{max}: 3330, 1738, 1651 cm^{-1}; UV λ_{max} (MeOH): 217 (ε 1.2×10^4) and 281 nm (2.3×10^3); FABMS: *m/z* 580, 582 ([M+H]$^+$); HRFABMS: *m/z* 580.2790, calcd for $C_{29}H_{43}N_3O_7{}^{35}Cl$ 580.2787; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4.

3.3.4. Seragamide D (4). Colorless glass, $[\alpha]_D^{23}$ +46 (*c* 0.075, $CHCl_3$); IR (neat) ν_{max}: 2290, 1734, 1665 cm^{-1}; UV λ_{max} (MeOH): 217 (ε 1.2×10^4) and 285 nm (2.6×10^3); FABMS: *m/z* 658 ($[M+H]^+$); HRFABMS: *m/z* 658.1989, calcd for $C_{28}H_{41}N_3O_7I$ 658.1975; ^1H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4.

3.3.5. Seragamide E (5). Colorless glass, $[\alpha]_D^{24}$ +33 (*c* 0.090, $CHCl_3$); IR (neat) ν_{max}: 3340, 1732, 1653 cm^{-1}; UV λ_{max} (MeOH): 218 (ε 1.1×10^4) and 285 nm (2.5×10^3); FABMS: *m/z* 688 ($[M+H]^+$); HRFABMS: *m/z* 688.2095, calcd for $C_{29}H_{43}N_3O_8I$ 688.2103; ^1H and ^{13}C NMR: Tables 3 and 4.

3.3.6. Seragamide F (6). Colorless glass, $[\alpha]_D^{24}$ +10 (*c* 0.02, $CHCl_3$); IR (neat) ν_{max}: 3350, 1716, 1684 cm^{-1}; UV λ_{max} (MeOH): 229 (ε 4.7×10^3) and 279 nm (1.4×10^3); FABMS: *m/z* 642 ($[M+H]^+$); HRFABMS: *m/z* 642.2946, calcd for $C_{34}H_{45}N_3O_7^{35}Cl$ 642.2906; ^1H NMR ($CDCl_3$): δ 0.85 (d, 3H, J=6.7 Hz; H-32), 1.06 (d, 3H, J=6.7 Hz; H-16), 1.08 (d, 3H, J=6.4 Hz; H-31), 1.17 (d, 3H, J=7.0 Hz; H-34), 1.17 (m, 1H; H-7), 1.33 (m, 1H; H-7), 1.58 (d, 3H, J=4.0 Hz; H-33), 1.92 (br d, 1H, J=15.6 Hz; H-3), 2.27 (m, 1H; H-6), 2.39 (dd, 1H, J=11.7, 15.6 Hz; H-3), 2.55 (m, 1H; H-2), 2.61 (dd, 1H, J=6.0, 15.3 Hz; H-10), 2.69 (dd, 1H, J=4.6, 15.3 Hz; H-10), 2.91 (dd, 1H, J=10.6, 14.7 Hz; H-18), 2.92 (s, 3H; H-17), 3.25 (dd, 1H, J=6.4, 14.7 Hz; H-18), 4.63 (m, 1H; H-8), 4.78 (m, 1H; H-5), 4.79 (m, 1H; H-15), 5.26 (m, 1H; H-11), 5.45 (dd, 1H, J=6.4, 10.6 Hz; H-13), 6.66 (d, 1H, J=7.0 Hz; 15-NH), 6.76 (d, 2H, J=8.8 Hz; H-27, 29), 6.90 (d, 1H, J=8.2 Hz; H-23), 6.99 (dd, 1H, J=1.8, 8.2 Hz; H-24), 7.06 (d, 2H, J=8.2 Hz; H-26, 30), 7.13 (d, 1H, J=1.8 Hz; H-20), 7.43 (br d, 1H, J=9.2 Hz; 11-NH).

3.3.7. Geodiamolide I (7). Colorless glass, $[\alpha]_D^{24}$ +37 (*c* 0.045, $CHCl_3$; lit.[14] +39.3); IR (neat) ν_{max}: 3330, 1734, 1684 cm^{-1}; UV λ_{max} (MeOH): 227 (ε 1.0×10^4) and 282 nm (2.5×10^3); ^1H and ^{13}C NMR ($CDCl_3$) data were identical with those reported.[14]

3.4. Acid hydrolysis of 1, 2 and 5

To a sample (0.3 mg) of **1** in a small glass tube was added 6 M HCl (0.5 mL). After sealing, it was heated at 110 °C for over night in an oven. The reaction mixture was cooled to ambient temperature and partitioned between EtOAc and H_2O. Each layer was concentrated to dryness under N_2 stream. The H_2O layer was used for making FDAA derivatives. The same procedure was applied for the hydrolysis of **2** and **5**.

3.5. Derivatization of amino acid with Marfey's reagent and HPLC analysis

To a 2 mL reaction vial containing 0.3 mg standard D-Ala in 10 μL of water was added 50 μL of *N*,α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA, Marfey's reagent) acetone solution (10 μg/mL) followed by 20 μL of 1 M $NaHCO_3$. The mixture was heated at 40 °C for 1 h. After cooling at room temperature it was neutralized by adding 10 μL of 2 M HCl solution. The resulting solution was filtered and concentrated by N_2 stream, and the resulting residue was stored in a freezer until HPLC analysis. The FDAA derivatives of L-Ala, D- and L-Thr, D- and L-*allo*-Thr, and D- and L-*N*-Me-Tyr were prepared in the same manner.

HPLC analyses of FDAA derivatives were carried out by using a reverse phase column (RP-18, Mightysil 250×10 mm) eluting with buffer–MeOH–MeCN (65/15/20) (flow rate: 0.8 mL/min) and detecting UV absorption at 340 nm. The buffer was prepared by adjusting 20 mM potassium hydroxide to pH 2–3 with 85 wt% phosphoric acid. Individual amino acid was identified by comparing retention times with those of FDAA derivatives of standard amino acids. The retention times were: L-Thr (24.5 min), D-Thr (40.6 min), L-*allo*-Thr (25.6 min), D-*allo*-Thr (29.9 min), L-Ala (39.2 min), D-Ala (42.8 min), L-*N*-Me-Tyr (43.0 min), and D-*N*-Me-Tyr (44.8 min). Derivatives of seragamide E (**5**) and standards were analyzed on a different column using the same solvents as above to give retention of L-Ser (14.6 min) and D-Ser (16.0 min).

3.6. Saponification of 1 and 11

Seragamide A (**1**, 10.8 mg) was dissolved in 1 mL of MeOH and 75 μL of 1 M KOH, and the mixture was kept stirring at room temperature for 4 h. The resulting solution was neutralized by adding 0.1 M HCl and partitioned between EtOAc and water. The organic layer was purified by preparative TLC (hexane/EtOAc, 1–6) on silica to give 3.0 mg (27%) of **12**.

Pure jaspamide (**11**) was isolated from a lipophilic extract of a yellow sponge *Jaspis* sp. collected off Makassar, Indonesia.[17] Its authentic nature was confirmed by NMR.[4,5] Jaspamide (**11**, 1.6 mg) was dissolved in 500 μL of MeOH and 100 μL of 1 M KOH, and the mixture was stirred at 40–50 °C for 5 h. Similar work-up as for **12** furnished 1.3 mg (79%) of **13**.

3.6.1. Compound 12. ^1H NMR ($CDCl_3$–CD_3OD, 9–1) δ 1.00 (d, 3H, J=6.8 Hz; H-15), 1.09 (d, 3H, J=6.1 Hz; H-25), 2.84 (dd, J=11.0, 14.6 Hz; H-17a), 2.98 (s, 3H; H-16), 3.25 (dd, J=5.6, 14.6 Hz; H-17b), 4.22 (m, 2H; H-10, 24), 4.61 (q, J=7.1 Hz; H-14), 5.33 (m; H-12), 6.76 (d, J=8.3 Hz; H-22), 7.00 (dd, J=1.9, 8.3 Hz; H-23), 7.46 (br s; H-19). For H-2 to 8-Me, see Table 2. ESIMS *m/z* 688 $[M-H]^-$, *m/z* 690 $[M+H]^+$, 712 $[M+Na]^+$.

3.6.2. Compound 13. ^1H NMR ($CDCl_3$–CD_3OD, 9–1) δ 0.78 (d, 3H, J=7.1 Hz; H-16), 2.60 (d, 2H, J=6.8 Hz; H-10), 2.94 (s, 3H; H-17), 3.18 (dd, J=10.7, 14.9 Hz; H-18a), 3.40 (m; H-18b), 4.59 (q, J=7.1 Hz; H-15), 5.22 (br; H-13), 5.26 (t, J=6.2 Hz; H-11), 6.60 (d, 2H, J=8.5 Hz; H-29, 31), 6.99 (d, 2H, J=8.5 Hz; H-28, 32), 7.04 (t, J=7.8 Hz; H-24), 7.11 (t, J=7.8 Hz; H-23), 7.27 (d, J=7.8 Hz; H-22), 7.48 (d, J=7.8 Hz; H-25). For H-2 to 8-Me, see Table 2. ESIMS *m/z* 725, 727 $[M-H]^-$, *m/z* 749, 751 $[M+Na]^+$, 771, 773 $[M+2Na-H]^+$.

3.7. Cell assay

NBT-T2 cells (BRC-1370) were purchased from Riken and cultured under a standard protocol using DMEM. Aliquots of test compounds in MeOH were added to culture wells

plated cells 1 day before. After incubating the sample wells for 1 or 2 days, the toxic effect of a drug was observed under a microscope. The IC_{50} values were measured by MTT colorimetric method. Cultured cells were inoculated into each well (24-well plate) with 1 mL of the medium. After preincubation (24 h, 37 °C, 5% CO_2), the sample solution was added to each well and it was incubated for 48 h. And then, MTT solution (100 μL, 5 mg/mL in PBS) was added to each well and incubated for 3 h. The medium was removed by aspiration. The residual formazan was dissolved in 1 mL of dimethylsulfoxide (DMSO). The resulting solution was diluted with DMSO (four times) and the absorbance was measured at 540 nm.

3.8. Actin polymerization/depolymerization assay

Prodan-labeled actin was prepared by the method described by G.M.[15] Polymerization of G-actin was carried out by making a Prodan-G-actin solution at 1 μM in F-buffer (100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM DTT, 0.1 mM ATP, 5 mM Tris, pH 8.0) in a cuvette. The time course of F-actin polymerization was recorded for 200 s using an excitation wavelength of 385 nm and emission at 490 nm in the presence of 1, 10, 20, 50, 100, and 200 nM of seragamide A (**1**), or without **1** as control. The depolymerization assay of Prodan-F-actin was performed by diluting a preformed 28 μM solution of Prodan-F-actin to 100 nM in G-buffer (0.2 mM $CaCl_2$, 1 mM DTT, 0.1 mM ATP, 5 mM Tris, pH 8.0). Time course experiments were similarly carried out as for polymerization in the presence of 0.05, 0.1, 1, and 10 nM of seragamide A (**1**) or without **1** as control.

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