

Masayoshi Arai

Jurnal 2012-1.pdf

Sources Overview

| | | |
|--------------------|---|-----|
| 18% | | |
| OVERALL SIMILARITY | | |
| 1 | Asami Hayashi, Masayoshi Arai, Mayumi Fujita, Motomasa Kobayashi. "Pyripyropenes, Fungal Sesquiterpenes Conjugated with .ALPHA... | 6% |
| | CROSSREF | |
| 2 | Masayoshi Arai, Kentaro Kamiya, Dayoung Shin, Hirokazu Matsumoto, Tomoya Hisa, Andi Setiawan, Naoyuki Kotoku, Motomasa Kobaya... | 3% |
| | CROSSREF | |
| 3 | Suna, H.. "Dysideamine, a new sesquiterpene aminoquinone, protects hippocampal neuronal cells against iodoacetic acid-induced cell d... | 3% |
| | CROSSREF | |
| 4 | Tabudravu, J.N.. "Axinellin C, a proline-rich cyclic octapeptide isolated from the Fijian marine sponge Stylotella aurantium", Tetrahedron, ... | 1% |
| | CROSSREF | |
| 5 | Jixun Zhan, Anna M. Burns, Manping X. Liu, Stanley H. Faeth, A. A. Leslie Gunatilaka. " Search for Cell Motility and Angiogenesis Inhibito... | 1% |
| | CROSSREF | |
| 6 | link.springer.com | 1% |
| | INTERNET | |
| 7 | Zivancev, Jelena. "Napredne spregnute tehnike u analizi ksenobiotika.", University of Novi Sad (Serbia), 2020 | <1% |
| | PUBLICATION | |
| 8 | H. Brückner, S. Haasmann, M. Langer, T. Westhauser, R. Wittner, H. Godel. "Liquid chromatographic determination of d- and l-amino aci... | <1% |
| | CROSSREF | |
| 9 | Shunji Aoki. "Bastadin 6, a spongean brominated tyrosine derivative, inhibits tumor angiogenesis by inducing selective apoptosis to en... | <1% |
| | CROSSREF | |
| 10 | Naoyuki Kotoku, Ryosuke Ishida, Hirokazu Matsumoto, Masayoshi Arai et al. "Biakamides A–D, Unique Polyketides from a Marine Spo... | <1% |
| | CROSSREF | |
| 11 | Pettit, G.R.. "Antineoplastic Agents 390. Isolation and Structure of Phakellistatin 12 from a Chuuk Archipelago Marine Sponge", Bioorg... | <1% |
| | CROSSREF | |
| 12 | Wu, Shwu-Li, Jui-Hsin Su, Yi Lu, Bo-Wei Chen, Chiung-Yao Huang, Zhi-Hong Wen, Yao-Haur Kuo, and Jyh-Horng Sheu. "Simplexins Jâ€" ... | <1% |
| | CROSSREF | |
| 13 | Yasuo Watanabe, Shunji Aoki, Daiki Tanabe, Andi Setiawan, Motomasa Kobayashi. "Cortistatins E, F, G, and H, four novel steroidal alkal... | <1% |
| | CROSSREF | |

Excluded search repositories:

None

Excluded from document:

- Bibliography
- Quotes
- Citations
- Small Matches (less than 10 words)

Excluded sources:

repository.lppm.unila.ac.id, internet, 63%

Masayoshi Arai, Yoshi Yamano, Mayumi Fujita, Andi Setiawan, Motomasa Kobayashi. "Stylissamide X, a new proline-rich cyclic octapeptide as an inhibitor of cell migration, from an Indonesian marine sponge of *Stylissa* sp.", *Bioorganic & Medicinal Chemistry Letters*, 2012, crossref, 61%

Arai, M.. "Stylissamide X, a new proline-rich cyclic octapeptide as an inhibitor of cell migration, from an Indonesian marine sponge of *Stylissa* sp.", *Bioorganic & Medicinal Chemistry Letters*, 20120215, crossref, 53%

Yoshi Yamano, Masayoshi Arai, Motomasa Kobayashi. "Neamphamide B, new cyclic depsipeptide, as an anti-dormant mycobacterial substance from a Japanese marine sponge of *Neamphius* sp.", *Bioorganic & Medicinal Chemistry Letters*, 2012, crossref, 10%

Yamano, Yoshi, Masayoshi Arai, and Motomasa Kobayashi. "Neamphamide B, new cyclic depsipeptide, as an anti-dormant mycobacterial substance from a Japanese marine sponge of *Neamphius* sp.", *Bioorganic & Medicinal Chemistry Letters*, 2012., crossref, 8%

Shunji Aoki, Mami Sanagawa, Yasuo Watanabe, Andi Setiawan, Masayoshi Arai, Motomasa Kobayashi. "Novel isomarabarian triterpenes, exhibiting selective anti-proliferative activity against vascular endothelial cells, from marine sponge *Rhabdastrella globostellata*", *Bioorganic & Medicinal Chemistry*, 2007, crossref, 8%

www.jstage.jst.go.jp, internet, 8%

Patamaporn Pruksakorn, Masayoshi Arai, Naoyuki Kotoku, Catherine Vilchèze et al. "Trichoderins, novel aminolipopeptides from a marine sponge-derived *Trichoderma* sp., are active against dormant mycobacteria", *Bioorganic & Medicinal Chemistry Letters*, 2010, crossref, 8%

www.j3.jstage.jst.go.jp, internet, 7%

linknovate.com, internet, 7%

www.jove.com, internet, 7%

www.internetchemie.info, internet, 6%

Masayoshi Arai, Kentaro Kamiya, Dayoung Shin, Hirokazu Matsumoto et al. "<i>N</i>-Methylniphatyne A, a New 3-Alkylpyridine Alkaloid as an Inhibitor of the Cancer Cells Adapted to Nutrient Starvation, from an Indonesian Marine Sponge of <i>Xestospongia</i> sp.", *CHEMICAL & PHARMACEUTICAL BULLETIN*, 2016, crossref, 5%

Xiao Wang, Brandon I. Morinaka, Tadeusz F. Molinski. " Structures and Solution Conformational Dynamics of Stylissamides G and H from the Bahamian Sponge ", *Journal of Natural Products*, 2014, crossref, 5%

Oh-Seok Kwon, Chang-Kwon Kim, Woong Sub Byun, Joonseok Oh et al. " Cyclopeptides from the Sponge ", *Journal of Natural Products*, 2018, crossref, 5%

Kentaro Takada, Akihiro Ninomiya, Masato Naruse, Yi Sun, Masayuki Miyazaki, Yuichi Nogi, Shigeru Okada, Shigeki Matsunaga. " Surugamides A–E, Cyclic Octapeptides with Four -Amino Acid Residues, from a Marine *Streptomyces* sp.: LC–MS-Aided Inspection of Partial Hydrolysates for the Distinction of - and -Amino Acid Residues in the Sequence ", *The Journal of Organic Chemistry*, 2013, crossref, 5%

Kai-Xuan Zhan, Wei-Hua Jiao, Fan Yang, Jing Li, Shu-Ping Wang, Yu-Shan Li, Bing-Nan Han, Hou-Wen Lin. " Reniochalistatins A–E, Cyclic Peptides from the Marine Sponge ", *Journal of Natural Products*, 2014, crossref, 5%

Pruksakorn, P.. "Trichoderins, novel aminolipopeptides from a marine sponge-derived *Trichoderma* sp., are active against dormant mycobacteria", *Bioorganic & Medicinal Chemistry Letters*, 20100615, crossref, 4%

Hideaki Suna, Masayoshi Arai, Yoshie Tsubotani, Asami Hayashi, Andi Setiawan, Motomasa Kobayashi. "Dysideamine, a new sesquiterpene aminoquinone, protects hippocampal neuronal cells against iodoacetic acid-induced cell death", *Bioorganic & Medicinal Chemistry*, 2009, crossref, 4%

www.mdpi.com, internet, 4%

Masayoshi Arai, Shunsuke Ishida, Andi Setiawan, Motomasa Kobayashi. "Haliclonaclamines, Tetracyclic Alkylpiperidine Alkaloids, as Anti-dormant Mycobacterial Substances from a Marine Sponge of *Haliclona* sp.", *Chemical and Pharmaceutical Bulletin*, 2009, crossref, 4%

Arai, Masayoshi, Shunsuke Ishida, Andi Setiawan, and Motomasa Kobayashi. "Haliclonaclamines, Tetracyclic Alkylpiperidine Alkaloids, as Anti-dormant Mycobacterial Substances from a Marine Sponge of *Haliclona* sp.", *CHEMICAL & PHARMACEUTICAL BULLETIN*, 2009., crossref, 4%

"Graphical contents list", *Bioorganic & Medicinal Chemistry Letters*, 20120215, crossref, 3%

Valentin Stonik, Sergey Fedorov. "Marine Low Molecular Weight Natural Products as Potential Cancer Preventive Compounds", *Marine Drugs*, 2014, crossref, 3%

Stonik, Valentin, and Sergey Fedorov. "Marine Low Molecular Weight Natural Products as Potential Cancer Preventive Compounds", *Marine Drugs*, 2014., crossref, 3%

Masayoshi Arai, Chisu Han, Yoshi Yamano, Andi Setiawan, Motomasa Kobayashi. "Aptamines, marine spongean alkaloids, as anti-dormant mycobacterial substances", *Journal of Natural Medicines*, 2014, crossref, 3%

Ting Huang, Yan Zou, Mao-cheng Wu, Qing-jie Zhao, Hong-gang Hu. "Total Synthesis of Proline-Rich Cyclic Octapeptide Stylissamide X", *Chemistry of Natural Compounds*, 2015, crossref, 3%

www.frontiersin.org, internet, 2%

pdfs.semanticscholar.org, internet, 2%

Daniela Giordano, Maria Costantini, Daniela Coppola, Chiara Lauritano et al. "Biotechnological Applications of Bioactive Peptides From Marine Sources", *Elsevier BV*, 2018, crossref, 2%

Masayoshi Arai, Yoshi Yamano, Mayumi Fujita, Andi Setiawan, Motomasa Kobayashi. "ChemInform Abstract: Stylissamide X (I), a New Proline-Rich Cyclic Octapeptide as an Inhibitor of Cell Migration, from an Indonesian Marine Sponge of *Stylissa* sp.", *ChemInform*, 2012, crossref, 1%

Christine Cychon, Gesine Schmidt, Matthias Köck. "Sequencing of cyclic peptides by NMR and MS techniques demonstrated on stylissamides A–F", *Phytochemistry Reviews*, 2013, crossref, 1%

Rabab Mohammed, Jiangnan Peng, Michelle Kelly, Mark. T. Hamann. " Cyclic Heptapeptides from the Jamaican Sponge ", *Journal of Natural Products*, 2006, crossref, 1%

Christine Cychon, Matthias Köck. " Stylissamides E and F, Cyclic Heptapeptides from the Caribbean Sponge ", *Journal of Natural Products*, 2010, crossref, 1%

Christine Cychon, Matthias Köck. " Stylistamides E and F, Cyclic Heptapeptides from the Caribbean Sponge ", Journal of Natural Products, 2010, crossref, 1%

www.aminer.org, internet, 1%

onlinelibrary.wiley.com, internet, 1%

coek.info, internet, 1%

ir.kib.ac.cn:8080, internet, 1%

Van-Tinh Nguyen, Zhong-Ji Qian, BoMi Ryu, Kil-Nam Kim et al. "Matrix metalloproteinases (MMPs) inhibitory effects of an octameric oligopeptide isolated from abalone *Haliotis discus hannai*", Food Chemistry, 2013, crossref, 0%

Wan-Yin Fang, Rajiv Dahiya, Hua-Li Qin, Rita Mourya, Sandeep Maharaj. "Natural Proline-Rich Cyclopolypeptides from Marine Organisms: Chemistry, Synthetic Methodologies and Biological Status", Marine Drugs, 2016, crossref, 0%

Rajiv Dahiya, Sunil Singh, Ajay Sharma, Suresh Chennupati, Sandeep Maharaj. "First Total Synthesis and Biological Screening of a Proline-Rich Cyclopeptide from a Caribbean Marine Sponge", Marine Drugs, 2016, crossref, 0%

Silvia Scarpato, Roberta Teta, Gerardo Della Sala, Joseph R. Pawlik, Valeria Costantino, Alfonso Mangoni. "New Tricks with an Old Sponge: Feature-Based Molecular Networking Led to Fast Identification of New Stylistamide L from *Stylissa caribica*", Marine Drugs, 2020, crossref, 0%

Mudit Mudit, Khalid A. El Sayed. "Cancer control potential of marine natural product scaffolds through inhibition of tumor cell migration and invasion", Drug Discovery Today, 2016, crossref, 0%

www.wjgnet.com, internet, 0%

res.mdpi.com, internet, 0%

Nguyen, Van-Tinh, Zhong-Ji Qian, BoMi Ryu, Kil-Nam Kim, Daekyung Kim, Young-Mog Kim, You-Jin Jeon, Won Sun Park, Il-Whan Choi, Geun Hyung Kim, Jae-Young Je, and Won-Kyo Jung. "Matrix metalloproteinases (MMPs) inhibitory effects of an octameric oligopeptide isolated from abalone *Haliotis discus hannai*", Food Chemistry, 2013., crossref, 0%

Mudit, Mudit, and Khalid A. El Sayed. "Cancer control potential of marine natural product scaffolds through inhibition of tumor cell migration and invasion", Drug Discovery Today, 2016., crossref, 0%

uclouvain.be, internet, 0%

jpkc.fudan.sh.cn, internet, 0%

cyberleninka.org, internet, 0%

IIT Delhi on 2013-07-08, submitted works, 0%

Univerza v Ljubljani on 2013-04-17, submitted works, 0%

www.academia.edu, internet, 0%

pt.scribd.com, internet, 0%

orca.cf.ac.uk, internet, 0%

inje.academia.edu, internet, 0%

aprat.fr, internet, 0%

anacor.com, internet, 0%

Capela, Rita Sofia Salvador Simoues. "Compostos hibridos Da Artemisinin: Direccinamento Para as Fases sanguinea e hepatica Do Parasita Da malaria.", Universidade de Lisboa (Portugal), 2021, publication, 0%

Kingston University on 2021-01-10, submitted works, 0%

Univerza v Ljubljani on 2013-04-19, submitted works, 0%

www.procetus.com, internet, 0%

www.researchgate.net, internet, 0%

www.nature.com, internet, 0%

Arai, Masayoshi, Yoshi Yamano, Andi Setiawan, and Motomasa Kobayashi. "Identification of the Target Protein of Agelasine D, a Marine Sponge Diterpene Alkaloid, as an Anti-dormant Mycobacterial Substance", ChemBioChem, 2014., crossref, 0%

Kingston University on 2021-01-11, submitted works, 0%

Kingston University on 2021-01-11, submitted works, 0%

King Saud University on 2015-07-04, submitted works, 0%

King Saud University on 2014-03-26, submitted works, 0%

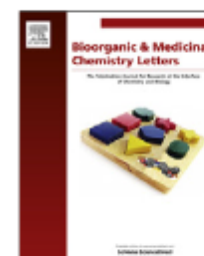
University of South Florida on 2013-12-10, submitted works, 0%

"Graphical contents list", Bioorganic & Medicinal Chemistry Letters, 20120201, crossref, 0%

"Graphical contents list", Bioorganic & Medicinal Chemistry Letters, 20120115, crossref, 0%

"Graphical contents list", Bioorganic & Medicinal Chemistry Letters, 20120101, crossref, 0%

cpb-us-e1.wpmucdn.com, internet, 0%



Stylissamide X, a new proline-rich cyclic octapeptide as an inhibitor of cell migration, from an Indonesian marine sponge of *Stylissa* sp.

Masayoshi Arai^{a,*}, Yoshi Yamano^a, Mayumi Fujita^a, Andi Setiawan^b, Motomasa Kobayashi^{a,*}

^a Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka 1-6, Suita, Osaka 565-0871, Japan

^b Department of Chemistry, Faculty of Science, Lampung University, Jl. Prof. Dr. Sumantri Brodjonegoro No. 1, Bandar Lampung 35145, Indonesia

ARTICLE INFO

Article history:

Received 6 September 2011

Revised 5 October 2011

Accepted 7 October 2011

Available online 13 October 2011

Keywords:

Stylissamide X

Marine sponge

Cancer

Migration

Metastasis

ABSTRACT

A new proline-rich cyclic octapeptide named stylissamide X¹⁰ was isolated from an Indonesian marine sponge of *Stylissa* sp. as an inhibitor of cell migration from the guidance of wound-healing assay. The chemical structure of stylissamide X² was determined on the basis of spectroscopic analysis, and stereostructure of the amino acids were deduced by Marfey's method. Compound 1 showed inhibitory activity against migration of HeLa cells in the ranges of 0.1–10 μ M concentration through both wound-healing assay and chemotaxicell chamber assay, while the cell viability was maintained more than 75% up to 10 μ M concentration of 1.

© 2011 Elsevier Ltd. All rights reserved.

Cancer metastasis is responsible for over 90% of cancer deaths¹ and consists of the multi-step process involving epithelial–mesenchymal transition (EMT), degradation of matrix, cell migration, and cell adhesion.² Then, cell migration is attracting much attention as one of the alternative strategies for development of anti-cancer chemotherapies. So far, several natural products such as withaferin A,³ prodigiosin,⁴ and migrastatin⁵ have been reported to inhibit cell migration.

In the course of our study of pharmaceutical valuable substances from marine organisms, we started a search for anti-cell migration substances and isolated a new proline-rich cyclic octapeptide named stylissamide X⁶ from an Indonesian marine sponge of *Stylissa* sp. on the basis of bioassay-guided separation. In this paper, the structure elucidation of stylissamide X (1) and its anti-cell migration activity are presented.

The dried marine sponge of *Stylissa* sp. 05A05 (500 g), which was collected in 2005 at Biak, Indonesia, was extracted with MeOH to obtain a MeOH extract. The MeOH extract (125 g), which showed anti-cell migration activity through wound-healing assay,⁷ was partitioned into a water–EtOAc mixture (1:1). The EtOAc soluble portion was further partitioned with hexane–90% aq MeOH mixture (1:1) to furnish a 90% MeOH extract (62 g) and hexane extract (36 g). On the guidance of bioassay, the 90% MeOH extract [62 g, minimum inhibitory concentration (MIC) of anti-cell

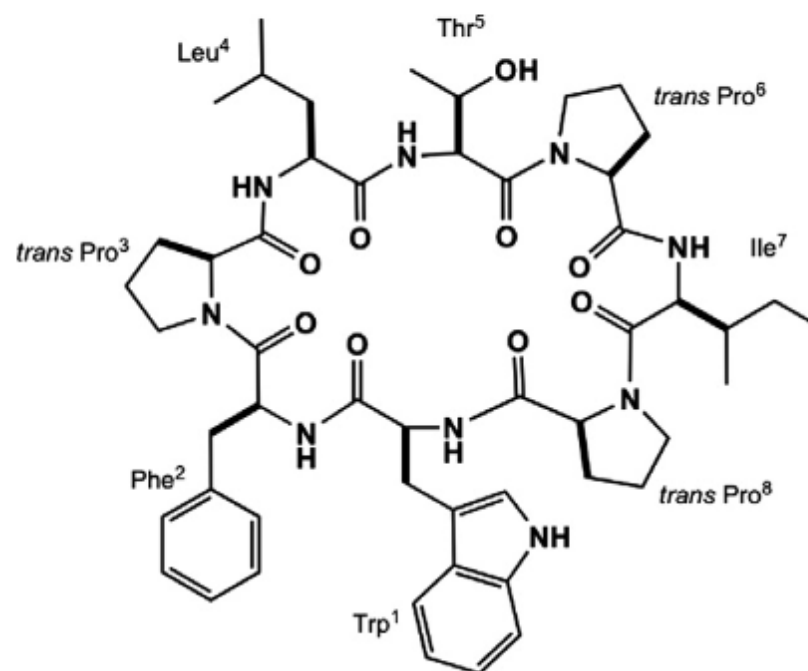


Figure 1. Chemical structure of stylissamide X (1).

migration activity = 10 μ g/mL] was further fractionated by SiO₂ gel column chromatography [CHCl₃/MeOH/H₂O = 65:3:1 (lower phase)] to give 10 fractions (Fr. M1–Fr. M10). The active Fr. M5 (3.6 g, MIC = 1.0 μ g/mL) was then separated by ODS column chromatography (MeOH–H₂O) to obtain five fractions (Fr. M51–Fr. M55). The active Fr. M53 (180 mg, MIC = 0.1 μ g/mL) was further

* Corresponding authors. Tel.: +81 66879 8215; fax: +81 66879 8219 (M.K.); tel./fax: +81 66879 8217 (M.A.).

E-mail addresses: araim@phs.osaka-u.ac.jp (M. Arai), kobayasi@phs.osaka-u.ac.jp (M. Kobayashi).

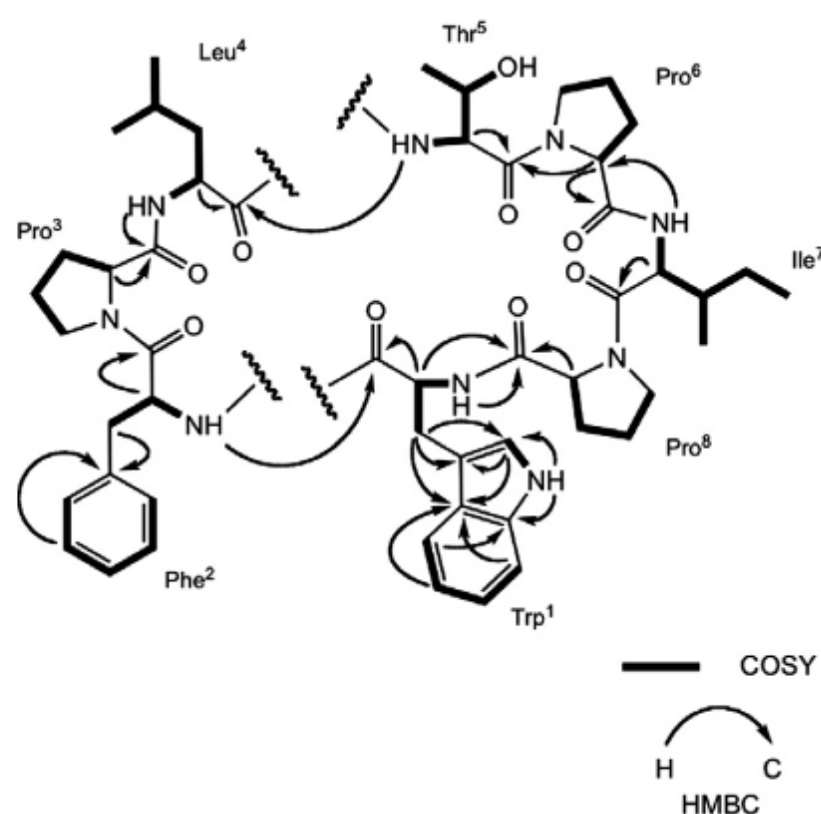


Figure 2. COSY and HMBC correlations for stylissamide X (1).

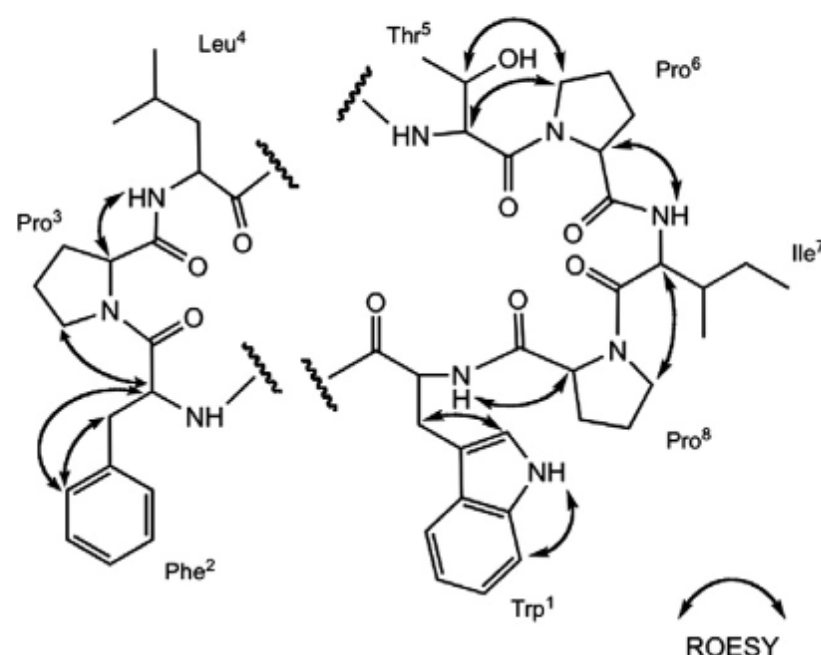


Figure 3. ROESY correlations for stylissamide X (1).

purified by ODS HPLC (Cosmosil MS-II, MeOH:H₂O = 8:2) to afford stylissamide X (1) (16 mg, 0.013% yield from the MeOH extract) (Fig. 1).

Stylissamide X (1) was obtained as a colorless solid. The ESI-TOF MS of 1 showed a pseudomolecular ion peak at m/z 974 $[M+Na]^+$. The molecular formula was determined as C₅₁H₆₉N₉O₉ by high-resolution (HR-) ESI-TOF MS. The IR absorptions at 3490, 3320, and 1670 cm⁻¹ suggested the presence of hydroxyl group, amino group, and amide carbonyl group, respectively. The observation of the signals at 168–170 ppm in the ¹³C NMR spectrum and the downfield signals (7.5–10.8 ppm) of the ¹H NMR spectrum indicated peptide structure of 1. To confirm the amino acid composition of 1, compound 1 was treated by dabsyl chloride following to acid hydrolysis.⁸ HPLC analysis of the dabsylated amino acids revealed that compound 1 contained three mol of Pro, one mol each of Leu, Ile, Thr, Trp, and Phe in the structure. The existing of eight amino acids was also confirmed by NMR analysis including TOCSY, HMQC, and HMBC experiments (Fig. 2). The connectivity of these amino acids was figured out by HMBC and ROESY analysis. As shown in Figure 3, the ROESY correlations between Trp¹-NH (δ_H 8.24) and Pro⁸-H α (δ_H 3.90); Phe²-H α (δ_H 5.00) and Pro³-H δ_1 (δ_H 3.90), Pro³-H δ_2 (δ_H 3.60); Pro³-H α (δ_H 4.00) and Leu⁴-NH (δ_H 8.70); Thr⁵-H α (δ_H 4.90) and Pro⁶-H δ_1 (δ_H 3.70); Thr⁵-H β (δ_H 4.10) and Pro⁶-H δ_2 (δ_H 3.57); Pro⁶-H α (δ_H 4.40) and Ile⁷-NH (δ_H 8.30); Ile⁷-H α (δ_H 4.20) and Pro⁸-H δ_1 (δ_H 3.96), Pro⁸-H δ_2 (δ_H 3.53) revealed the presence of the sequences of Phe²-Pro³-Leu⁴ and Thr⁵-Pro⁶-Ile⁷-Pro⁸-Trp¹. In addition, the HMBC correlations (Fig. 2) between Phe²-NH (δ_H 8.10) and Trp¹-CO (δ_C 170.1); Trp¹-H α (δ_H 3.68) and Trp¹-CO; Thr⁵-NH (δ_H 7.50) and Leu⁴-CO (δ_C 170.1); Leu⁴-H α (δ_H 3.56) and Leu⁴-CO proved that stylissamide X (1) has *cyclo*-(Trp-Phe-Pro-Leu-Thr-Pro-Ile-Pro) structure. Stereostructure of amino acids was confirmed by HPLC analysis of the derivatized amino acids by 5-fluoro-2,4-dinitrophenyl-L-alanine-amide (Marfey's reagent).⁹ The result of HPLC analysis indicated that all the constituting amino acids in 1 were found to be L-amino acids. In addition, geometry of the peptidic linkages at the proline residues was figured out on the basis of the ¹³C chemical shift difference between the proline β and γ carbons ($\Delta\delta_{\beta-\gamma}$). The small differences (Pro³ $\Delta\delta_{\beta-\gamma}$ = 5.0, Pro⁶ $\Delta\delta_{\beta-\gamma}$ = 4.4, Pro⁸ $\Delta\delta_{\beta-\gamma}$ = 3.6) indicated that all the proline peptide bonds were *trans* geometry.^{10,11,12c} The ROESY correlations between Phe²-H α and Pro³-H δ_1 , Pro³-H δ_2 ; Ile⁷-H α and Pro⁸-H δ_1 , Pro⁸-H δ_2 ; Thr⁵-H α and Pro⁶-H δ_1 , Pro⁶-H δ_2 also supported the *trans* geometry of these proline peptidic linkages. Taken together, the gross structure of stylissamide X (1) was elucidated as shown in Figure 1. All the proton- and carbon-signals were assigned as shown

Table 1
¹H and ¹³C NMR data for stylissamide X (1)

| Residue | δ_C^a | δ_H^b | Residue | δ_C^a | δ_H^b |
|------------------|--------------|----------------------|----------------------------|--------------|---------------------|
| Trp ¹ | | | Leu ⁴ | | |
| 4 | 57.0 | 3.68 (m) | α | 52.9 | 3.56 (m) |
| β | 23.4 | 3.64 (m) | β | 36.1 | 1.67 (m), 2.15 (m) |
| 2 | 123.5 | 3.16 (dd, 12.5, 3.0) | γ | 24.7 | 1.47 (m) |
| 3 | 111.2 | 6.96 (s) | δ_1 CH ₃ | 23.3 | 0.88 (d, 7.0) |
| 4 | 136.0 | | δ_2 CH ₃ | 18.5 | 0.85 (d, 6.5) |
| 5 | 111.3 | 7.30 (d, 8.0) | CO | 170.1 | |
| 6 | 120.8 | 7.00 (t, 7.5) | NH | | 8.70 (d, 6.0) |
| 7 | 118.1 | 6.92 (dd, 8.0, 7.0) | Thr ⁵ | | |
| 8 | 117.9 | 7.40 (d, 7.5) | α | 56.3 | 4.90 (dd, 9.0, 3.0) |
| 9 | 127.0 | | β | 67.6 | 4.10 (m) |
| CO | 170.1 | | β OH | | 5.50 (d, 12.5) |
| NH (indole) | | 10.8 (d, 1.5) | γ CH ₃ | 21.0 | 0.94 (d, 6.0) |
| NH | | 8.24 (d, 6.5) | CO | 168.9 | |
| | | | NH | | 7.50 (d, 9.5) |

(continued on next page)

Table 1 (continued)

| Residue | δ_C^a | δ_H^b | Residue | δ_C^a | δ_H^b |
|------------------|--------------|----------------------------------|--------------------------|--------------|---|
| Phe ² | | | Pro ⁶ | | |
| α | 51.0 | 5.00 (m) | α | 59.2 | 4.40 (dd, 8.5, 4.0) |
| β | 36.4 | 2.96 (d, 14.5, 4.0) | β | 29.1 | 1.95 (m), 2.06 (m) |
| | | 2.86 (dd, 14.5, 9.0) | γ | 24.7 | 1.74 (m) ^c , 1.84 (m) ^c |
| 1 | 138.1 | | δ_1 | 47.0 | 3.70 (m) |
| 2/6 | 129.2 | 7.25 (m) | δ_2 | | 3.57 (m) |
| 3/5 | 127.8 | 7.21 (m) | CO | 170.6 | |
| 4 | 125.9 | 7.10 (t, 7.5) | Ile ⁷ | | |
| CO | 169.8 | | α | 53.9 | 4.20 (t, 9.5) |
| NH | | 8.10 (d, 9.5) | β | 35.0 | 1.98 (m) |
| Pro ³ | | | β CH ₃ | 24.6 | 0.86 (d, 7.0) |
| α | 60.6 | 4.00 (t, 7.5) | γ | 14.4 | 1.03 (m), 1.30 (m) ^c |
| β | 29.7 | 1.74 (m) ^c , 2.13 (m) | δ CH ₃ | 9.7 | 0.65 (t, 7.5) |
| γ | 24.7 | 1.84 (m) ^c , 2.05 (m) | CO | 170.4 | |
| δ_1 | 47.8 | 3.90 (m) ^c | NH | | 8.30 (d, 8.0) |
| δ_2 | | 3.60 (m) | Pro ⁸ | | |
| CO | 170.7 | | α | 60.9 | 3.90 (m) ^c |
| | | | β | 28.4 | 1.94 (m), 2.00 (m) |
| | | | γ | 24.8 | 1.30 (m) ^c , 1.77 (m) |
| | | | δ_1 | 47.5 | 3.96 (m) |
| | | | δ_2 | | 3.53 (m) |
| | | | CO | 170.9 | |

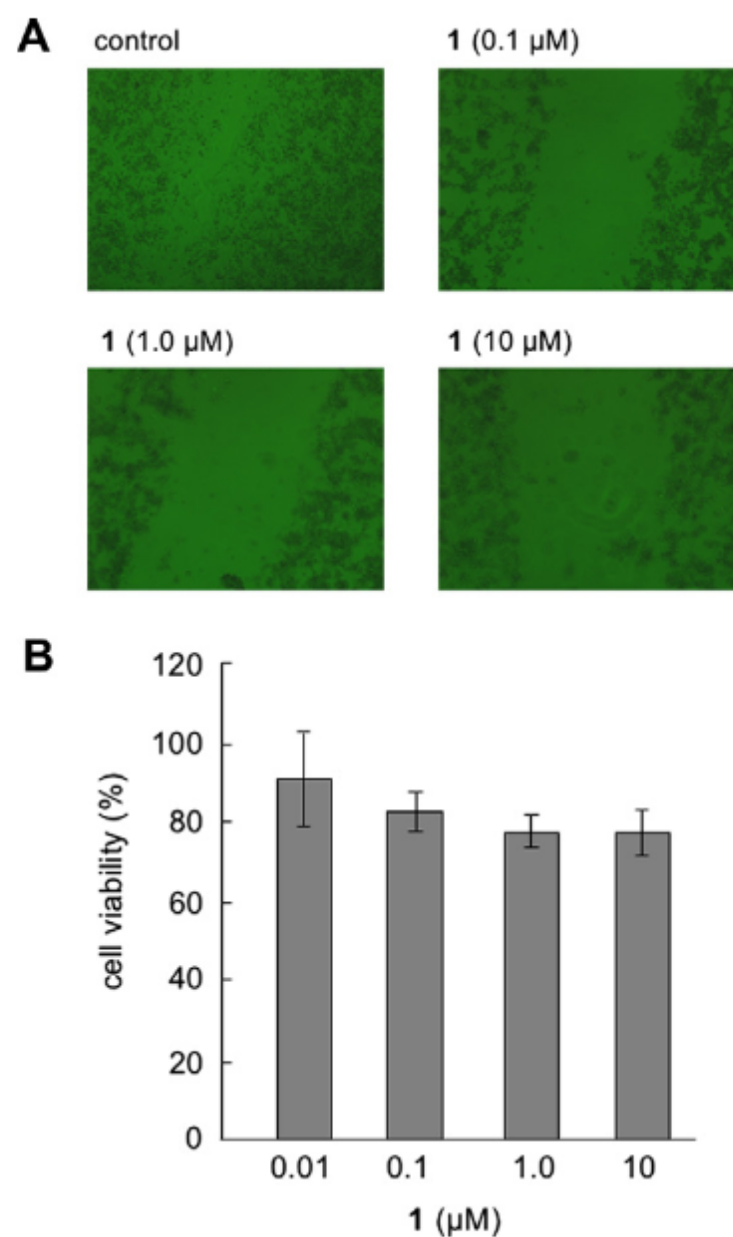
^a ¹³C NMR: δ_C (ppm), (150 MHz, DMSO-d₆).^b ¹H NMR: δ_H (ppm, *J* in Hz), (600 MHz, DMSO-d₆).^c Overlapping resonances.

Figure 4. Anti-migration activity and anti-proliferative activity of stylissamide X (**1**). (A) Anti-migration activity of **1** by wound-healing assay, (B) anti-proliferative activity of **1** by MTT method.

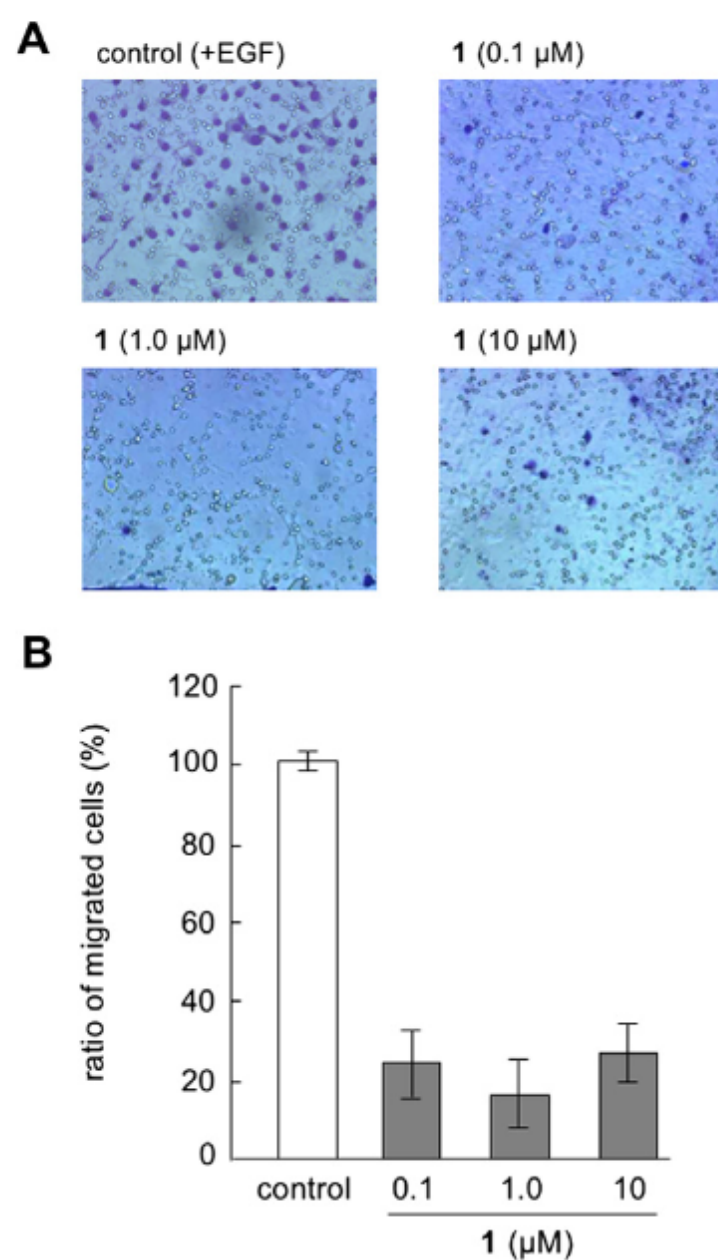


Figure 5. Effect of stylissamide X (**1**) on the migration of HeLa cells induced by EGF. HeLa cells (3×10^4 cells) were treated with the indicated concentrations of **1** and stimulated with EGF (50 ng/mL). After 24 h, the migrated cells to the reverse side of the membrane filter were counted in the six different microscopic fields. Representative figure of the migrated HeLa cells are shown (A). Data were shown by percentage based on the migrated cell number of the EGF-stimulated HeLa cells (B).

in Table 1. Styliissamide X (**1**) is structurally classified to a group of the proline-rich cyclic peptides, which are characterized to consist of seven or eight amino acids containing two or three proline residues and have been isolated from various genera of marine sponges such as *Axinella*,¹² *Stylotella*,¹³ *Phakellia*,¹⁴ *Hymeniacidon*,¹⁵ and *Stylissa*.¹⁶ So far, these cyclic peptides have been found to exhibit moderate cytotoxic activity and weak antimicrobial activity.

Styliissamide X (**1**) inhibited migration of HeLa cells in the concentration ranging from 0.1 μ M to 10 μ M through wound-healing assay, whereas cell viability of HeLa cells were maintained more than 75% up to 10 μ M of **1** as shown in Figure 4. In addition, styliissamide X (**1**) also inhibited EGF-induced migration of HeLa cells¹⁷ from 0.1 μ M to 10 μ M concentration of **1** through chemotaxicell chamber assay (Fig. 5). Further detailed evaluation of the anti-migration activity of tumor cells is under way.

Acknowledgements

This study was financially supported by the Hoansha Foundation, the Uehara Memorial Foundation and Grant-in-Aid for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References and notes

- Germanov, E.; Berman, J. N.; Guernsey, D. L. *Int. J. Mol. Med.* **2006**, *18*, 1025.
- Gavert, N.; Ben-Ze'ev, A. *Trends Mol. Med.* **2008**, *14*, 199.
- Lee, J.; Hahm, E. R.; Singh, S. V. *Carcinogenesis* **2010**, *31*, 1991.
- Zhang, J.; Shen, Y.; Liu, J.; Wei, D. *Biochem. Pharmacol.* **2005**, *69*, 407.
- Nakae, K.; Yoshimoto, Y.; Sawa, T.; Homma, Y.; Hamada, M.; Takeuchi, T.; Imoto, M. *J. Antibiot.* **2000**, *53*, 1130.
- Styliissamide X (**1**): Colorless solid. $[\alpha]_D^{20} -77$ ($c = 0.2$, CHCl_3). IR ν_{max} (KBr) cm^{-1} : 3490, 3320, 1670. UV λ_{max} (MeOH) nm (ϵ): 209 (7700). ESI-MS: m/z 974 $[\text{M}+\text{Na}]^+$. High resolution ESI-MS: Calcd for $\text{C}_{51}\text{H}_{69}\text{N}_9\text{O}_9\text{Na}$: m/z 974.5116. Found 974.5107. ^1H NMR (600 MHz, $\text{DMSO}-d_6$, δ_{H}), ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$, δ_{C}) spectra: as shown in Table 1.
- (a) Liang, C. C.; Park, A. Y.; Guan, J. L. *Nat. Protoc.* **2007**, *2*, 329; (b) Wound-healing assay was performed according to the method described by Liang, et al. [Ref. 7a]. Briefly, the suspension of HeLa cells in DMEM supplemented with 10% FBS were plated into each well of 96-well plates (1×10^5 cells/well/100 μL), and were incubated for 24 h in a humidified atmosphere of 5% CO_2 at 37 $^\circ\text{C}$ to allow the forming of confluent monolayer. After 24 h, the monolayer was scratched with a pipette tip, washed with culture medium to remove floating cells, and photographed. Then, the cells were treated with testing samples for 24 h. The cells were photographed again to evaluate whether the cells treated with testing samples were able to migrate from the cutting edge of wound or not. The anti-migration activity of testing samples was determined as a minimum concentration of testing samples to inhibit the migration of cells completely.
- Styliissamide X (**1**) (0.5 mg) were treated with 2 mL of 1.2 N HCl aq and heated at 110 $^\circ\text{C}$ for 12 h. The reaction mixture was dried under reduced pressure to obtain crude amino acids. The crude amino acids from styliissamide X (**1**) and authentic amino acids were dissolved in 40 μL of 50 mM sodium bicarbonate (pH 8.1) in glass tubes respectively. Then, 80 μL of freshly prepared 4-dimethylaminoazobenzene-4'-sulfonyl chloride (DABS-Cl) solution (4 mM in acetonitrile) was added to each sample. The samples were sealed with caps and Parafilm. The samples were then heated at 70 $^\circ\text{C}$ for 10 min. After dabsylation, the samples were analyzed by reversed-phase HPLC under the following condition; Cosmosil 5C₁₈ AR, (4.6 mm i.d. \times 250 mm), a 50 min linear gradient from acetonitrile–25 mM sodium acetate buffer (pH 6.5) = 3:7 to 1:1, 30 $^\circ\text{C}$, 1 mL/min, and detection at 436 nm. Authentic dabsylated Thr, Pro, Ile, Leu, Trp, and Phe were eluted with retention times of 24.8, 30.8, 37.2, 38.4, 40.4, and 42.4 min, respectively.
- The crude amino acids from styliissamide X (**1**) and L- and D-authentic amino acids were dissolved in 100 μL of 0.5 M sodium bicarbonate in 3-mL screw-cap glass tubes, respectively. Then, 100 μL of 5-fluoro-2,4-dinitrophenyl-L-alanine amide (L-FDAA, Marfey's reagent, 10 mg/mL in acetone) was added to each sample. The samples were sealed with caps and incubated at 70 $^\circ\text{C}$ for 60 min. After addition of 20 μL of 1 N HCl aq, the reaction mixture was diluted with methanol to suitable volumes (10–15 fold dilution). An aliquot of the L-FDAA derivatives was analyzed by HPLC under the following condition; Cosmosil μ NAP, (4.6 mm i.d. \times 250 mm), a 80 min linear gradient from acetonitrile– H_2O containing 0.1% TFA = 1:9 to 1:1, 1.5 mL/min, and detection at 340 nm. Authentic derivatized L-Thr, D-Thr, L-Pro, D-Pro, L-Ile, D-Ile, L-Leu, D-Leu, L-Phe, D-Phe, L-Trp, and D-Trp were eluted with retention times of 39.6, 43.2, 47.6, 51.2, 62.8, 68.8, 63.6, 68.8, 66.4, 71.6, 64.8, and 68.4 min, respectively.
- (a) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 1719; (b) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.
- McDonald, L. A.; Foster, M. P.; Phillips, D. R.; Ireland, C. M.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 4616.
- (a) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A.; Rutzler, K. C. *J. Med. Chem.* **1991**, *34*, 3339; (b) Pettit, G. R.; Gao, F.; Cerny, R. L.; Doubek, D. L.; Tackett, L. P.; Schmidt, J. M.; Chapuis, J. C. *J. Med. Chem.* **1994**, *37*, 1165; (c) Randazzo, A.; Piaz, F. D.; Orru, S.; Debitus, C.; Roussakis, C.; Pucci, P.; Paloma, L. G. *Eur. J. Org. Chem.* **1998**, 2659.
- (a) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Erickson, K. L.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Bakus, G. J. *J. Org. Chem.* **1992**, *57*, 7217; (b) Tabudravu, J.; Morris, L. A.; Kettenes-van den Bosch, J. J.; Jaspars, M. *Tetrahedron Lett.* **2001**, *42*, 9273; (c) Tabudravu, J. N.; Morris, L. A.; Kettenes-van den Bosch, J. J.; Jaspars, M. *Tetrahedron* **2002**, *58*, 7863.
- (a) Pettit, G. R.; Cichacz, Z.; Barkoczy, J.; Dorsaz, A. C.; Herald, D. L.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Brune, D. C.; Cerny, R. L.; Hooper, J. N. A.; Bakus, G. J. *J. Nat. Prod.* **1993**, *56*, 260; (b) Pettit, G. R.; Tan, R.; Williams, M. D.; Tackett, L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2869; (c) Pettit, G. R.; Xu, J. P.; Cichacz, Z. A.; Williams, M. D.; Dorsaz, A. C.; Brune, D. C.; Boyd, M. R.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2091; (d) Pettit, G. R.; Tan, R.; Ichihara, Y.; Williams, M. D.; Doubek, D. L.; Tackett, L. P.; Schmidt, J. M.; Cerny, R. L.; Boyd, M. R.; Hooper, J. N. A. *J. Nat. Prod.* **1995**, *58*, 961.
- (a) Kobayashi, J.; Tsuda, M.; Nakamura, T.; Mikami, Y.; Shigemori, H. *Tetrahedron* **1993**, *49*, 2391; (b) Tsuda, M.; Shigemori, H.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1993**, *49*, 6785; (c) Tsuda, M.; Sasaki, T.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 4667.
- (a) Mohammed, R.; Peng, J.; Kelly, M.; Hamann, M. T. *J. Nat. Prod.* **2006**, *69*, 1739; (b) Schmidt, G.; Grube, A.; Kock, M. *Eur. J. Org. Chem.* **2007**, 4103; (c) Cychon, C.; Kock, M. *J. Nat. Prod.* **2010**, *73*, 738.
- A polycarbonate filter of the inner chamber (Chemotaxicell chamber, 8 μm) was soaked in fibronectin solution (1.3 $\mu\text{g}/\text{mL}$) for 1 h at 37 $^\circ\text{C}$ and dried in vacuo. Then, HeLa cells (3.0×10^5 cells) were suspended in FBS free-DMEM containing the indicated concentration of **1** and seeded in the inner chamber. The inner chamber was put into the outer chamber (24-well plate), which was filled with FBS free-DMEM containing Epidermal Growth Factor (EGF) (5 $\mu\text{g}/\text{mL}$). After 24 h incubation at 37 $^\circ\text{C}$, the non-migrated cells on the upper surface of the filter were removed by wiping with cotton swabs, and the filter was fixed with 70% EtOH and stained with Giemsa. The cells, which migrated through the filter to the reverse side, were counted manually at six different areas under a microscope. Data were shown by percentage based on the migrated cell number of EGF-stimulated HeLa cells.