The Journal of Antibiotics (2017) 70, 607–610

www.nature.com/ja

ORIGINAL ARTICLE

Simamycin (5′-*O*-geranyluridine): a new prenylated nucleoside from *Streptomyces* sp.

Yasuhiro Igarashi[1], Takayuki Kyoso[1], Youngju Kim[1] and Tsutomu Oikawa[2]

A new nucleoside modified by prenylation, simamycin (1), was isolated from the culture broth of a soil-derived *Streptomyces* sp. Its structure was determined by spectroscopic analysis and chemical synthesis as 5′-*O*-geranyluridine. Compound 1 induced differentiation of preadipocytes into matured adipocytes.
The Journal of Antibiotics (2017) **70,** 607–610; doi:10.1038/ja.2016.163; published online 18 January 2017

INTRODUCTION

Despite the substantial amounts of studies on its secondary metabolites, *Streptomyces* is still believed to be the most prolific source of novel bioactive compounds. In fact, up to 40% of known microbial metabolites are derived from this group.[1] However, it is noteworthy that the number of metabolites isolated is far below the number of gene clusters for secondary metabolite biosynthesis present in the genome of *Streptomyces* species.[2,3] In our screening for structurally unique or rare secondary metabolites from *Streptomyces*, we isolated plant hormone-like spiroacetals of polyketide origin,[4] a linear polyketide δ-lactone with antimetastatic property,[5] macrocyclic polyketides with a biosynthetically unprecedented heterocyclic ring structure[6] and methylphenylalanine derivatives with adipocyte differentiation promoting activity.[7] Further attempts to obtain new bioactive compounds from *Streptomyces*, a soil-derived strain TP-A0872, was chosen for chemical investigation, which led to the isolation of a new geranylated nucleoside, simamycin (**1**; Figure 1).

RESULTS AND DISCUSSION

The producing strain TP-A0872 was isolated from a soil sample collected in Kochi, Japan, and identified as a member of the genus *Streptomyces* on the basis of 16S rRNA gene sequence analysis. The whole culture broth of strain TP-A0872 cultured in A-3M liquid medium was extracted with 1-butanol. HPLC/UV analysis of the extract and dereplication using our in-house metabolite database suggested the presence of an unknown peak showing a UV absorption band at 263 nm. HPLC/UV-guided purification from the extract led to the isolation of a new geranylated nucleoside, **1**. Subsequent biological evaluation elucidated that **1** induces the differentiation of preadipocytes into matured adipocytes. We herein describe the isolation, structure determination and biological activity of **1**.

Compound **1** was obtained as optically active colorless needles that gave an $[M+H]^+$ pseudomolecular ion at *m/z* 381.2027 appropriate for the molecular formula of $C_{19}H_{29}N_2O_6$ (7 degrees of unsaturation). This molecular formula was corroborated by ^1H and ^{13}C NMR spectral data (Table 1). The IR absorptions at 1694 and 1664 cm^{-1} suggested the presence of carbonyl functionalities. One- and 2D NMR data of **1** revealed the presence of 19 carbons, which were assigned to four quaternary sp^2 carbons including two oxygenated carbons, four proton-bearing sp^2 carbons, six oxygenated sp^3 carbons, two aliphatic methylenes and three methyls.

From the ^1H-^1H COSY spectrum, five proton-bearing fragments were established: H-15/H-16/H-17, H-12/H-13, H-10/H-11, H-7/H-8 and H-5/H-6. The first and the second fragments were joined at C-14 on the basis of HMBC correlations from the methyl protons H-20 to C-13, C-14 and C-15. This fragment was expanded to include a three-carbon fragment C-19/C-18/C-21 on the basis of HMBC correlations from H-19 and H-21 to C-17 and C-18, and to one another, providing a geranyl moiety. The third and the fourth fragments were connected at C-9 by HMBC correlations from H-8 to C-9 and C-10, H-9 to C-8 and C-11, and H-10 and H-11 to C-9, establishing connectivity from C-7 to C-11. Furthermore, HMBC correlations from H-7 to C-10 and H-10 to C-7 connected C-7 and C-10 via an ether linkage, to confirm a pentofuranosyl residue. The last fragment H-5/H-6 was extended to include two carbonyl carbons C-2 and C-4, and the latter carbon was connected to C-5 by HMBC correlations from H-5 and H-6 to C-4. As the molecular formula suggested the presence of two nitrogen atoms in **1**, analysis of ^1H-^{15}N long-range correlations became necessary to locate their positions. In the ^1H-^{15}N HMBC spectrum, both H-5 and H-6 showed a cross-peak to a nitrogen at 156.3 p.p.m. and H-5 to a nitrogen at 155.7 p.p.m. These correlation data, together with ^{13}C and ^{15}N chemical shifts, established this chromophore as pyrimidine-2,4(1*H*,3*H*)-dione, namely uracil. Finally, three

[1]Department of Biotechnology, Biotechnology Research Center, Toyama Prefectural University, Toyama, Japan and [2]Department of Nutritional Biochemistry, School of Nutrition and Dietetics, Kanagawa University of Human Services, Kanagawa, Japan
Correspondence: Professor Y Igarashi, Department of Biotechnology, Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan.
E-mail: yas@pu-toyama.ac.jp
This paper is dedicated to Professor Dr Satoshi Ōmura for his Nobel Prize in Physiology or Medicine 2015.
Received 21 October 2016; revised 1 December 2016; accepted 6 December 2016; published online 18 January 2017

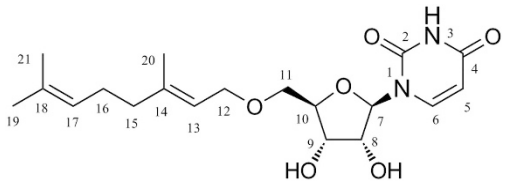


Figure 1 Structure of simamycin (**1**).

Table 1 ^{1}H and ^{13}C NMR data for **1** in $CDCl_3$

Position	δ_H *mult (J in Hz)*[a]	δ_C[b]	*HMBC*[c]
2		151.6	
4		164.2	
5	5.66 d (8.0)	102.2	4, 6
6	8.00 d (8.0)	141.0	2, 4, 5, 7
7	5.92 d (3.0)	90.5	2, 6, 8, 10
8	4.23	75.8	9, 10
9	4.21	84.4	8, 11
10	4.22	70.7	7
11	3.76 d (10.5)	68.7	9, 10, 12
	3.60 d (10.5)		9, 10, 12
12	4.04	67.9	11, 13, 14
13	5.29 t (6.5)	120.2	12, 15, 20
14		141.3	
15	2.05	39.8	13, 14, 17
16	2.10	26.6	17
17	5.08 t (6.5)	123.9	16, 19, 21
18		131.5	
19	1.73 s	25.9	17, 18, 21
20	1.68 s	16.6	13, 14, 15
21	1.60 s	17.9	17, 18, 19

[a]Recorded at 500 MHz.
[b]Recorded at 125 MHz.
[c]HMBC correlations are from proton(s) stated to the indicated carbon.

substructures were joined by analysis of HMBC data. HMBC correlations from H-11 and H-12 to one another connected the geranyl group at C-11 of the pentose unit via an ether linkage, whereas the uracil moiety was connected N-1 to the anomeric carbon (C-7) of the pentose unit by HMBC correlations from H-7 to C-2 and C-6, and H-6 to C-7. Thus, the gross structure of **1** was determined as shown in Figure 2.

As the signal overlapping of H-8, H-9 and H-10 in the ^{1}H NMR spectrum did not allow the stereochemical assignment of the pentose unit by NOE and *J*-based analysis, **1** was derivatized to bis-*p*-bromobenzoate (**2**) by the treatment with *p*-bromobenzoyl chloride in pyridine. The above mentioned proton signals were separated each other in the ^{1}H NMR spectrum of **2**. NOESY correlations were detected for H-8/H-9, H-7/H-10, H-2/H-8 and H-9/H-11 (Figure 3). These correlation data allowed the assignment of the pentose unit to possess a ribose configuration, thereby establishing **1** as 5′-*O*-geranyluridine.

To determine the absolute configuration of the ribose unit, **1** was synthesized starting from commercially available natural (+)-enantiomer of uridine in three steps. The *cis*-diol of uridine was protected as dimethylacetonide,[8] which was then *O*-alkylated using geranyl bromide in the presence of NaH to give **4**.[9] Geranylation at the 5-position of ribose was confirmed by an HMBC correlation from H-12 to C-11. Additional evidence for the alkylation site was the HMBC correlations from 3-NH (δ_H 9.58) to C-4 (δ_C 163.6) and C-5 (δ_C 102.0). The acetonide protective group was removed by treating **4** in HCl to afford **1** (Scheme 1). Optical rotation of the synthetic **1** was $[\alpha]_D^{24}$ +16 (*c* 0.1, $CHCl_3$), which was in good accordance with that of natural **1**, $[\alpha]_D^{24}$ +13 (*c* 0.1, $CHCl_3$). The absolute configuration of **1** was thus determined as depicted in Figure 1.

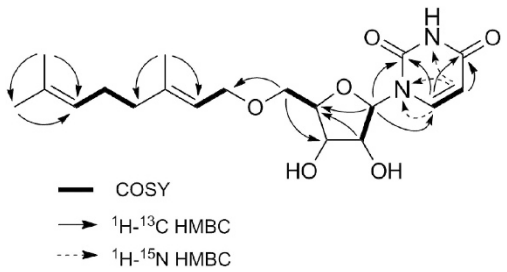


Figure 2 COSY and key HMBC correlations for **1**.

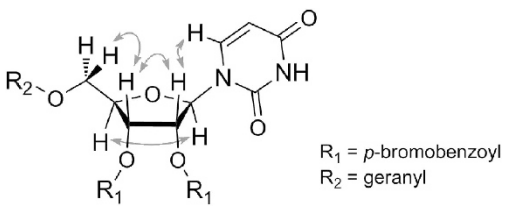


Figure 3 Key NOESY correlations for **2**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Compound **1** was not active against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in the antimicrobial assay (MIC $>50\,\mu g\,ml^{-1}$). It was also inactive in cytotoxicity assay (IC_{50} $>50\,\mu M$ against MCF7 human breast cancer cells). However, **1** was found to induce differentiation of murine ST-13 preadipocyte cells into matured adipocyte cells.[10] Maturation of adipocytes leads to the secretion of adiponectin, which downregulates the blood glucose level.[11] About 50% of preadipocytes were differentiated to adipocytes with accumulation of lipid droplets by the treatment with 40 μM of **1** for 11 days (Figure 4). JBIR-68 in which the double bond of uridine of **1** is hydrogenated was reported to inhibit influenza proliferation[12] but we had no access to this assay.

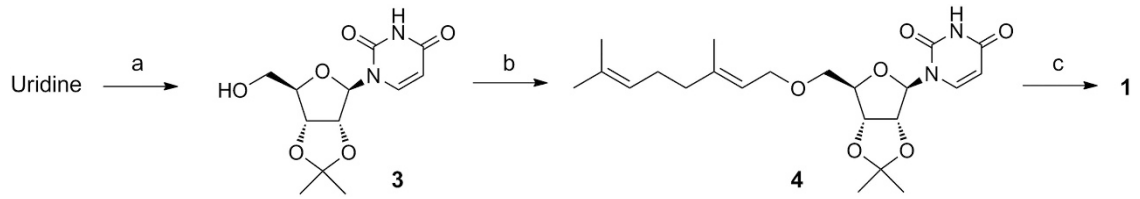
CONCLUSION

In summary, we found **1**, a new nucleoside modified by *O*-prenylation with a geranyl group from a soil-derived *Streptomyces* by UV-spectroscopic screening. Prenylation of nucleosides is very rare in nature. To date, JBIR-68 (Takagi *et al.*[12]) and farnesides A and B[13] are known as a secondary metabolite of *Streptomyces*. JBIR-68 is a dihydrouridine derivative in which the hydroxy group at five-position of ribose is modified with a geranyl group. Farnesides are also dihydrouridine derivatives but an oxygenated farnesyl group is connected to the hydroxy group at five-position of the ribose moiety.

EXPERIMENTAL PROCEDURE

General experimental procudures

Optical rotations were measured using a Jasco P-1030 polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were recorded on a Hitachi U-3210 spectrophotometer (Hitachi-High-Technologies Co., Tokyo, Japan). IR spectra were recorded on a Shimadzu FT-IR-300 spectrophotometer (Shimadzu Corp., Kyoto, Japan). NMR spectra were obtained on a Bruker AVANCE II 500 spectrometer (Bruker Biospin K. K., Yokohama, Japan). HR-FAB-MS and HR-ESI-MS were measured on a JEOL JMS-HX110A spectrometer (JEOL Ltd., Tokyo, Japan) and a Bruker micrOTOF (Bruker Daltonics K. K., Yokohama, Japan), respectively.



Scheme 1 Synthesis of **1**. Reagents: (**a**) 2,2-dimethoxypropane, TsOH; (**b**) geranylbromide, NaH; (**c**) dil HCl.

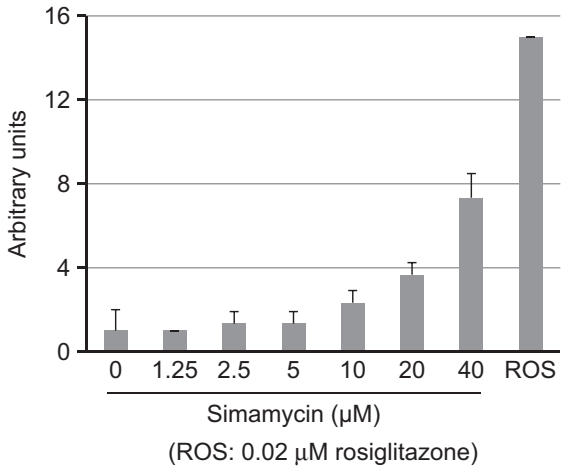


Figure 4 Adipocyte differentiation induced by **1**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Microorganism

The actinomycete, *Streptomyces* sp. TP-A0872, was isolated from a soil sample collected in Kochi, Japan. The isolated strain was identified as *Streptomyces* on the basis of 99.9% similarity in the 16S rRNA gene sequence (1463 nucleotides; DDBJ accession number LC192168) to *Streptomyces xanthophaeus* NRRL B-5414^T (accession number JOFT01000080).

Fermentation

Strain TP-A0872 cultured on a slant agar medium was inoculated into 500 ml K-1 flasks, each containing 100 ml of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract (Kyokuto Pharmaceutical Industrial, Co., Ltd, Tokyo, Japan) 0.2%, Tryptone (Difco Laboratories, Sparks, MD, USA) 0.5%, K_2HPO_4 0.1%, $MgSO_4{\cdot}7H_2O$ 0.05% and $CaCO_3$ 0.3% (pH 7.0). The flasks were cultivated on a rotary shaker (200 r.p.m.) at 30 °C for 4 days. The seed culture (3 ml) was transferred into 500 ml K-1 flasks each containing 100 ml of the production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein) 1.5%, yeast extract 0.3% and Diaion HP-20 (Mitsubishi Chemical Co., Yokohama, Japan) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were cultured on a rotary shaker (200 r.p.m.) at 30 °C for 7 days.

Extraction and isolation

The whole culture broth of strain TP-A0872 (3 l) was extracted with 1-butanol (100 ml per flasks) on a rotary shaker (200 r.p.m.) for 1 h. The mixture was centrifuged at 6000 r.p.m. for 10 min and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent *in vacuo* provided 5.23 g of extract, which was subjected to silica gel column chromatography with a step gradient of $CHCl_3$-MeOH (16:1, 8:1, 4:1, 2:1, 1:1 and 0:1 v/v). Concentration of fraction 2 ($CHCl_3$-MeOH = 8:1) provided 1.1 g of brown powders, which was further purified by HPLC separation with a gradient of MeCN-0.15% KH_2PO_4 buffer (pH 3.5) (0–6 min: 40%; 6–20 min: 40–50%; 15 ml min^{-1}). Compound **1** was eluted at 17.8 min. Fraction was pooled and concentrated under reduced pressure and the remaining aqueous solution was extracted with EtOAc. The organic layer was concentrated to give **1** (8.8 mg) as a colorless solid.

Simamycin (5-O′-geranyluridine, 1). Colorless needles; mp 95–96 °C; $[\alpha]_D^{24}$ +13 (*c* 0.10, $CHCl_3$); UV λ_{max} (log ε) 263 nm (3.90); IR (KBr) ν_{max} 3377, 1694, 1664 cm^{-1}; For ^{1}H and ^{13}C NMR data, see Table 1. ^{15}N NMR ($CDCl_3$) δ 156.3 (N-1), 155.7 (N-3) (chemical shifts were indirectly determined from the projection of ^{1}H-^{15}N HMBC spectrum); HR-FABMS $[M+H]^+$ *m/z* 381.2027 (calcd for $C_{19}H_{30}N_2O_6$, 381.2025).

Bis-*p*-bromobenzoate of 1 (2)

To a solution of **1** (3.0 mg, 7.9 μmol) in dry CH_2Cl_2 (1.2 ml) and dry pyridine (0.6 ml) were added *N*,*N*-dimethyl-4-aminopyridine (a trace amount) and *p*-bromobenzoyl chloride (3.5 mg, 16 μmol) at 0 ~ 5 °C. After stirring for 20 h at room temperature, the reaction mixture was diluted with ice water and extracted with EtOAc. The organic layer was successively washed with sat. $CuSO_4$ solution, sat. $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane-EtOAc, 10:1 ~ 1:1) to give **2** (1.0 mg, 17% yield) as a light yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (1H, s), 5.76 (1H, d, *J* = 8.2 Hz, H-5), 7.99 (1H, d, *J* = 8.2 Hz, H-6), 6.53 (1H, d, *J* = 7.7 Hz, H-7), 5.62 (1H, dd, *J* = 7.7, 5.6 Hz, H-8), 5.73 (1H, m, H-9), 4.48 (1H, br.s, H-10), 3.81 (1H, dd, *J* = 9.4, 1.7 Hz, H-11), 3.72 (1H, dd, *J* = 9.4, 1.7 Hz, H-11), 4.18 (1H, dd, *J* = 11.6, 6.8 Hz, H-12), 4.13 (1H, dd, *J* = 11.6, 7.2 Hz, H-12), 5.37 (1H, t, *J* = 6.5 Hz, H-13), 2.31 (2H, m, H-15), 2.09 (2H, m, H-16), 5.02 (1H, t, *J* = 6.6 Hz, H-17), 1.67 (3H, s, H-19), 1.73 (3H, s, H-20), 1.57 (3H, s, H-21), 7.84 (2H, d, *J* = 8.5 Hz), 7.46 (2H, d, *J* = 8.5 Hz), 7.73 (2H, d, *J* = 8.5 Hz), 7.58 (2H, d, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.3 (C-2), 162.4 (C-4), 103.3 (C-5), 140.0 (C-6), 85.9 (C-7), 74.2 (C-8), 73.5 (C-9), 83.1 (C-10), 69.2 (C-11), 67.9 (C-12), 119.4 (C-13), 142.3 (C-14), 39.6 (C-15), 26.4 (C-16), 123.6 (C-17), 131.98 (C-18), 25.7 (C-19), 16.5 (C-20), 17.7 (C-21), 127.3, 127.7, 129.07, 129.13, 131.2, 131.3, 132.0, 132.1, 164.5, 164.8; HR-FABMS $[M+Na]^+$ *m/z* 767.0580 (calcd for $C_{33}H_{34}N_2O_8Br_2Na$, 756.0579).

5-*O*-Geranyl-2,3-*O*-isopropylidene-1-uracil-β-D-ribofuranoside (4)

To a solution of uridine (700 mg, 2.87 mmol) in dry *N*,*N*-dimethylformamide (70 ml) were added 2,2-dimethoxypropane (7 ml, 57 mmol) and *p*-toluenesulfonic acid monohydrate (70 mg, 0.37 mmol) at room temperature. After stirring for 18 h, the reaction was quenched by adding saturated $NaHCO_3$ solution to the reaction mixture until the pH became 7.0. The reaction mixture was diluted with distilled water and loaded onto an HP-20 column and the column was washed with distilled water. The column was then eluted with MeOH and the eluent was concentrated under reduced pressure to give 2,3-*O*-isopropylidene-1-uracil-β-D-ribofuranoside (**3**, 500 mg, 61% yield). Spectroscopic data were in good agreement with reported values.[9]

To a suspension of **3** (120 mg, 0.42 mmol) in a mixture of dry benzene (1.45 ml) and dry 1,4-dioxane (0.45 ml) was added NaH (40% purity, 60 mg, 2.5 mmol) under argon atmosphere. The mixture was heated at 80 ~ 100 °C for 30 min with stirring. After cooling to the ambient temperature, geranyl bromide (0.17 ml, 0.89 mmol) was added and the reaction mixture was heated at 90 ~ 95 °C for 3 h. The reaction was quenched by adding water at 0 ~ 5 °C and the mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was applied to silica gel column chromatography (*n*-hexane:EtOAc = 16:1 ~ 1.1) to give **4** (39 mg, 22% yield): ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.58 (1H, s), 7.68 (1H, d, *J* = 8.0 Hz), 5.95 (1H, d, *J* = 3.0 Hz), 5.67 (1H, d, *J* = 8.0 Hz), 5.29

(1H, t, $J=5.5$ Hz), 5.06 (1H, t, $J=5.5$ Hz), 4.79 (1H, dd, $J=6.0$, 3.0 z), 4.78 (1H, dd, $J=4.0$, 2.5 Hz), 4.39 (1H, dd, $J=3.5$, 3.0 Hz), 4.03 (1H, d, $J=7.0$ Hz), 3.69 (1H, dd, $J=8.0$, 2.5 Hz), 3.60 (1H, dd, $J=8.0$, 3.5 Hz), 2.09 (2H, m), 2.05 (2H, m), 1.68 (3H, s), 1.66 (3H, s), 1.06 (3H, s), 1.59 (3H, s), 1.36 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.5, 17.7, 25.3, 25.7, 26.3, 27.2, 39.6, 67.8, 69.8, 81.8, 85.1, 85.6, 92.6, 102.0, 114.1, 119.9, 123.7, 131.8, 141.1, 141.3, 150.3, 163.6; HR-ESITOFMS $[M+Na]^+$ 443.2153 (calcd for $C_{22}H_{32}N_2O_6Na$, 443.2153).

Synthetic 1

A stirred solution of **4** (9.4 mg, 22 μmol) in 95% EtOH (3 ml) and 0.1 M HCl (1 ml) was heated at 70 ~ 80 °C for 4 h. After cooling to the ambient temperature, 0.1 M NaOH solution was added to adjust the pH to 7.0. The reaction mixture was then extracted with EtOAc and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by preparative HPLC in the same manner described for natural simamycin to yield 6.3 mg of **1** (74% yield): colorless solid; $[\alpha]^{24}_D$ +16 (*c* 0.10, $CHCl_3$); ^{1}H and ^{13}C NMR data were in good agreement with those for natural **1**; HR-ESITOFMS $[M+Na]^+$ 403.1840 (calcd for $C_{19}H_{28}N_2O_6Na$, 403.1839).

Biological assays. Adipocyte differentiation assay[10], antimicrobial assay[14] and cytotoxic assay[14] were carried out according to the procedures previously described.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Bérdy, J. Bioactive microbial metabolites. *J. Antibiot.* **58**, 1–26 (2005).
2 Ōmura, S. *et al.* Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. *Proc. Natl Acad. Sci. USA* **98**, 12215–12220 (2001).
3 Ohnishi, Y. *et al.* Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. *J. Bacteriol.* **190**, 4050–4060 (2008).
4 Igarashi, Y., Iida, T., Yoshida, R. & Furumai, T. Pteridic acids A and B, novel plant growth promoters with auxin-like activity from *Streptomyces hygroscopicus* TP-A0451. *J. Antibiot.* **55**, 764–767 (2002).
5 Igarashi, Y. *et al.* Absolute configuration of pterocidin, a potent inhibitor of tumor cell invasion from a marine-derived *Streptomyces*. *Tetrahedron Lett.* **53**, 654–656 (2012).
6 Igarashi, Y. *et al.* Alchivemycin A, a bioactive polycyclic polyketide with an unprecedented skeleton from *Streptomyces* sp. *Org. Lett.* **12**, 3402–3405 (2010).
7 Igarashi, Y. *et al.* Jomthonic acid A, a modified amino acid from a soil-derived *Streptomyces*. *J. Nat. Prod.* **75**, 986–990 (2012).
8 Zhang, W. *et al.* Nine enzymes are required for assembly of the pacidamycin group of peptidyl nucleoside antibiotics. *J. Am. Chem. Soc.* **133**, 5240–5243 (2011).
9 Holý, A. *et al.* 5′-*O*-Alkyl-5-fluorouridines: synthesis and biological activity. *Collect. Czech. Chem. Commun.* **52**, 1589–1608 (1987).
10 Kunimasa, K. *et al.* Identification of nobiletin, a polymethoxyflavonoid, as an enhancer of an adiponectin secretion. *Bioorg. Med. Chem. Lett.* **19**, 2062–2064 (2009).
11 Kadowaki, T. *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116**, 1784–1792 (2006).
12 Takagi, M. *et al.* Anti-influenza virus compound from *Streptomyces* sp. RI18. *Org. Lett.* **12**, 4664–4666 (2010).
13 Ilan, E. Z. *et al.* Farnesides A and B, sesquiterpenoid nucleoside ethers from a marine-derived *Streptomyces* sp., strain CNT-372 from Fiji. *J. Nat. Prod.* **76**, 1815–1818 (2013).
14 Igarashi, Y. *et al.* Maklamicin, an antibacterial polyketide from an endophytic *Micromonospora* sp. *J. Nat. Prod.* **74**, 670–674 (2011).