

ORIGINAL ARTICLE

Pentaceciliides, new inhibitors of lipid droplet formation in mouse macrophages produced by *Penicillium cecidicola* FKI-3765-1: II. Structure elucidation

Hiroyuki Yamazaki¹, Satoshi Ōmura² and Hiroshi Tomoda¹

The structures of pentaceciliides, new inhibitors of lipid droplet formation in mouse macrophages produced by *Penicillium cecidicola* FKI-3765-1, were elucidated by spectroscopic studies, including various NMR experiments. Pentaceciliides have a common pentacyclic meroterpene core, which contains an aromatic ring and a δ -lactone ring.

The Journal of Antibiotics (2009) 62, 207–211; doi:10.1038/ja.2009.19; published online 20 March 2009

Keywords: pentaceciliides; structure elucidation; fungal metabolites; lipid droplet formation

INTRODUCTION

Three new compounds, designated pentaceciliides A to C (Figure 1) were isolated as inhibitors of lipid droplet formation in mouse macrophages from the culture broth of *P. cecidicola* FKI-3765-1.¹ The taxonomy of the producing strain, fermentation, isolation and biological properties of pentaceciliides were described in an earlier paper.¹ In this study, the physicochemical properties and structure elucidation of pentaceciliides are described.

RESULTS

Physicochemical properties

The physicochemical properties of pentaceciliides A to C are summarized in Table 1. They have a similar pattern with absorption maxima at 214–219 nm, 273–274 nm and 309–310 nm in UV spectra. IR absorption at 1619–1745 cm⁻¹ and 3401–3434 cm⁻¹ suggested the presence of carbonyl and hydroxy groups in their structures. These data indicated that they share a similar skeleton.

Structure elucidation of pentaceciliide C

The molecular formula of pentaceciliide C was determined to be C₂₇H₃₄O₈ on the basis of HRESI-TOF-MS measurement (Table 1). The ¹³C NMR spectrum (in CDCl₃) showed 27 resolved signals, which were classified into six methyl carbons, four methylene carbons, two *sp*³ methine carbons, one *sp*² methine carbon, three oxygenated *sp*³ methine carbons, two *sp*³ quaternary carbons, one oxygenated *sp*³ quaternary carbon, three *sp*² quaternary carbons, two oxygenated *sp*² quaternary carbons and three carbonyl carbons by analysis of the DEPT and heteronuclear single quantum coherence (HSQC) spectra.

The ¹H NMR spectrum (in CDCl₃) displayed 33 proton signals, one of which was suggested to be a hydroxyl proton (δ 11.08), as reported in thailandolides.² Taking the molecular formula into consideration, the presence of another hydroxy proton was suggested. The connectivity of proton and carbon atoms was established by the ¹³C–¹H HSQC spectrum (Table 2). Analyses of ¹H–¹H COSY revealed the presence of partial structures I to IV, as shown in Figure 2. Furthermore, ¹³C–¹H long-range couplings of ²*J* and ³*J* observed in the ¹³C–¹H HMBC spectrum gave the following linkages (Figure 3a): (1) Cross-peaks from H₂-7' (δ 2.70, 2.85) to C-1' (δ 110.7), C-2' (δ 139.3) and C-3' (δ 102.2), from OH-4' (δ 11.08) to C-3', C-4' (δ 162.3) and C-5' (δ 103.5) and from H-5' (δ 6.30) to C-1', C-3', C-4' and C-6' (δ 159.1) indicated that a phenol skeleton connects the partial structure I at C-2'. Furthermore, the findings that the chemical shift of C-8' (δ 74.7) corresponds to an oxygenated carbon and the OH-4' proton (δ 11.08) shifted to a lower field because of a hydrogen bonding indicated that C-3' and C-8' are connected through an ester bond, which form δ -lactone. This was also supported by the IR absorption (1619–1666 cm⁻¹). Although observation of a cross-peak from H-8' to C-10' (δ 169.9) was important and simple to show the presence of δ -lactone, the cross-peak was not observed because the dihedral angle between H-8' and C-10' is 90°. Therefore, the coupling constant in HMBC experiment was changed from *J*_{C–H}=8.0 Hz to *J*_{C–H}=3.0 Hz. As a result, the long-range coupling of ⁴*J* from H-5' to C-10' was observed, supporting the presence of δ -lactone. (2) Cross-peaks from H₂-11 (δ 2.52) to C-8 (δ 79.0), from H₃-12 (δ 1.23) to C-7 (δ 71.8), C-8 and C-9 (δ 43.0), from H-7 (δ 4.11) to C-8 and C-9, from H-9 (δ 2.23) to C-8, C-10 (δ 36.2) and C-15 (δ 24.6), from H₂-6

¹Graduate School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo, Japan and ²Kitasato Institute for Life Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo, Japan

Correspondence: Professor H Tomoda, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.

E-mail: tomudah@pharm.kitasato-u.ac.jp

Received 15 January 2009; revised 6 February 2009; accepted 19 February 2009; published online 20 March 2009

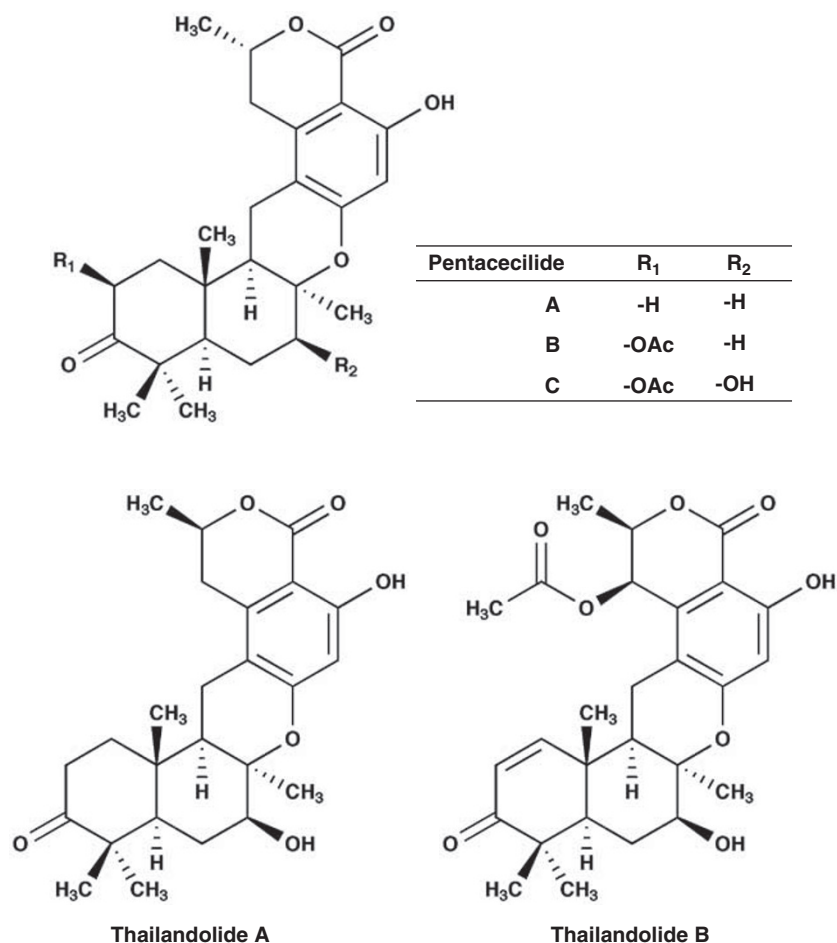


Figure 1 Structures of pentacecilides A to C and thailandolides A and B.

Table 1 Physicochemical properties of pentacecilides A, B and C

	Pentacecicide A	Pentacecicide B	Pentacecicide C
Appearance	White crystalline solid	White crystalline solid	White crystalline solid
Molecular weight	412	470	486
Molecular formula	C ₂₅ H ₃₂ O ₅	C ₂₇ H ₃₄ O ₇	C ₂₇ H ₃₄ O ₈
HRESI-TOF-MS (<i>m/z</i>)			
Calcd:	435.2147 (M+Na) ⁺	493.2225 (M+Na) ⁺	509.2151 (M+Na) ⁺
Found:	435.2141 (M+Na) ⁺	493.2202 (M+Na) ⁺	501.2162 (M+Na) ⁺
UV (MeOH) λ _{max} nm (ε)	219 (18647), 274 (11846), 309 (4194)	219 (33055), 274 (18226), 309 (4784)	214 (54432), 273 (32736), 310 (4947)
[α] _D ²⁰	-4.38° (c=0.38, CHCl ₃)	-32.6° (c=0.68, CHCl ₃)	-32.3° (c=0.48, CHCl ₃)
IR (KBr) ν _{max} (cm ⁻¹)	3401, 1697, 1662, 1465, 1380	3440, 1747, 1727, 1666, 1475	3434, 1745, 1666, 1619, 1473

(δ 1.86, 2.18) to C-10, from H₃-15 (δ 1.54) to C-9, C-10 and C-1 (δ 41.1), from H-5 (δ 1.82) to C-4 (δ 48.3), C-10 and C-15, from H₂-1 (δ 1.88, 2.20) to C-3 (δ 207.8), C-5, C-10 and C-15, from H₃-13 (δ 1.14) to C-3, C-4, C-5 and C-14 (δ 25.7), from H₃-14 (δ 1.20) to C-3, C-4, C-5 and C-13 (δ 20.8) and from H-2 (δ 5.63) to C-3 showed the presence of a 3-oxo-decalin skeleton containing the partial structures II to IV. (3) Cross-peaks from H-2 and H₃-17 (δ 2.17) to C-16 (δ 170.3) showed that an acetoxy group is connected to C-2. The chemical shift of C-7 (δ 71.8) and the molecular formula showed the presence of a hydroxy group. (4) The finding that cross-peaks were

observed from H-11 to C-1', C-2' and C-6' and that the chemical shifts of C-8 (δ 79.0) and C-6' (δ 159.1) correspond to an oxygenated carbon indicated that a phenol and a decalin ring are connected by a pyran ring. The pentacyclic structure was found to consist of a six-membered lactone, a phenol, a pyran and a decalin ring. Thus, the structure of pentacecicide C was elucidated as shown in Figure 1. The structure satisfied the degree of unsaturation and the molecular formula. Furthermore, all chemical shifts, except for C-2 in pentacecicide C, were comparable with those reported for thailandolide A.²

Table 2 ^1H and ^{13}C NMR chemical shift of pentaceciliides A, B and C

No.	Pentaceciliide A		Pentaceciliide B		Pentaceciliide C	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	31.7	1.67 m 2.06 m	40.7	1.85 m 2.18 m	41.1	1.88 m 2.20 m
2	33.7	2.44 m 2.68 m	72.6	5.59 (12.0, 7.0)	72.2	5.63 (12.0, 7.0)
3	219.6	—	208.6	—	207.8	—
4	47.2	—	48.6	—	48.3	—
5	48.1	1.88 m	48.0	1.77 m	45.6	1.82 m
6	18.5	1.58 m	17.7	1.68 m 1.75 m	26.7	1.86 m 2.18 m
7	33.7	2.08 m 2.24 m	36.6	2.10 m	71.8	4.11 dd (10.0, 3.0)
8	78.3	—	78.1	—	79.0	—
9	44.8	1.86 m	48.5	1.88 m	43.0	2.23 m
10	35.4	—	36.6	—	36.2	—
11	20.3	2.44 m	21.2	2.44 dd (10.0, 2.5)	20.7	2.52 m
12	25.5	1.35 s	24.0	1.26 s	21.2	1.23 s
13	20.0	1.09 s	21.3	1.18 s	20.8	1.20 s
14	29.3	1.12 s	26.3	1.16 s	25.7	1.14 s
15	23.0	0.96 s	24.9	1.39 s	24.6	1.54 s
16	—	—	170.3	—	170.3	—
17	—	—	21.0	2.17 s	21.0	2.17 s
1'	110.5	—	110.9	—	110.7	—
2'	139.2	—	139.3	—	139.3	—
3'	101.7	—	101.9	—	102.2	—
4'	162.5	—	162.6	—	162.3	—
5'	103.3	6.27 s	103.5	6.27 s	103.5	6.30 s
6'	160.4	—	160.2	—	159.1	—
7'	32.0	2.72 dd (17.0, 11.0) 2.84 dd (17.0, 3.5)	32.1	2.70 dd (17.0, 11.0) 2.85 dd (17.0, 3.5)	31.8	2.70 dd (17.0, 11.0) 2.85 dd (17.0, 3.5)
8'	75.0	4.64 m	74.9	4.62 m	74.7	4.62 m
9'	21.2	1.54 d (7.0)	21.2	1.55 d (7.0)	20.9	1.55 d (7.0)
10'	170.5	—	170.3	—	169.9	—
4'-OH	—	11.08 s	—	11.07 s	—	11.08 s

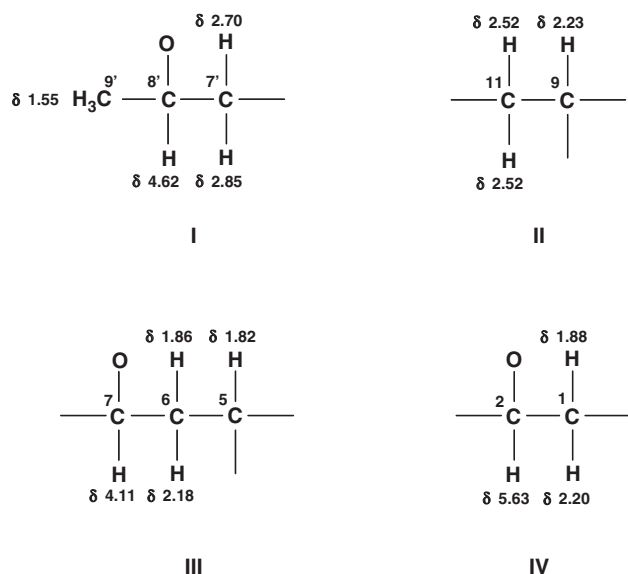


Figure 2 Partial structures of pentaceciliide C.

Structure elucidation of pentaceciliide B

The molecular formula $\text{C}_{27}\text{H}_{34}\text{O}_7$ of pentaceciliide B is smaller by one oxygen atom than that of pentaceciliide C. Comparison of the ^1H NMR spectra between pentaceciliides B and C indicated that the oxygenated sp^3 methine proton (H-7) in pentaceciliide C is replaced by methylene protons (δ 2.10) in pentaceciliide B. In fact, analyses of the ^1H - ^1H COSY revealed the presence of the partial structure V containing the replaced part (Figure 3b). The partial structure V was also confirmed by observing cross-peaks from H_3 -12 (δ 1.26) to C-7 (δ 36.6), C-8 (δ 78.1) and C-9 (δ 48.5), from H_2 -7 (δ 2.10) to C-8, from H-9 (δ 1.88) to C-8, C-10 (δ 36.6) and C-15 (δ 24.9), from H_2 -6 (δ 1.68, 1.75) to C-10, from H_3 -15 (δ 1.39) to C-9 and C-10 and from H-5 (δ 1.77) to C-10 in ^{13}C - ^1H HMBC experiments (Figure 3b). Taken together, the structure of pentaceciliide B was elucidated as 7-dehydroxy pentaceciliide C (Figure 1).

Structure elucidation of pentaceciliide A

The molecular formula $\text{C}_{25}\text{H}_{32}\text{O}_5$ of pentaceciliide A is smaller by $\text{C}_2\text{H}_2\text{O}_3$ than that of pentaceciliide C. Comparison of the ^1H NMR spectra of pentaceciliides A and B showed that the methyl protons (H_3 -17) disappear and the oxygenated sp^3 methine proton (H-2) is

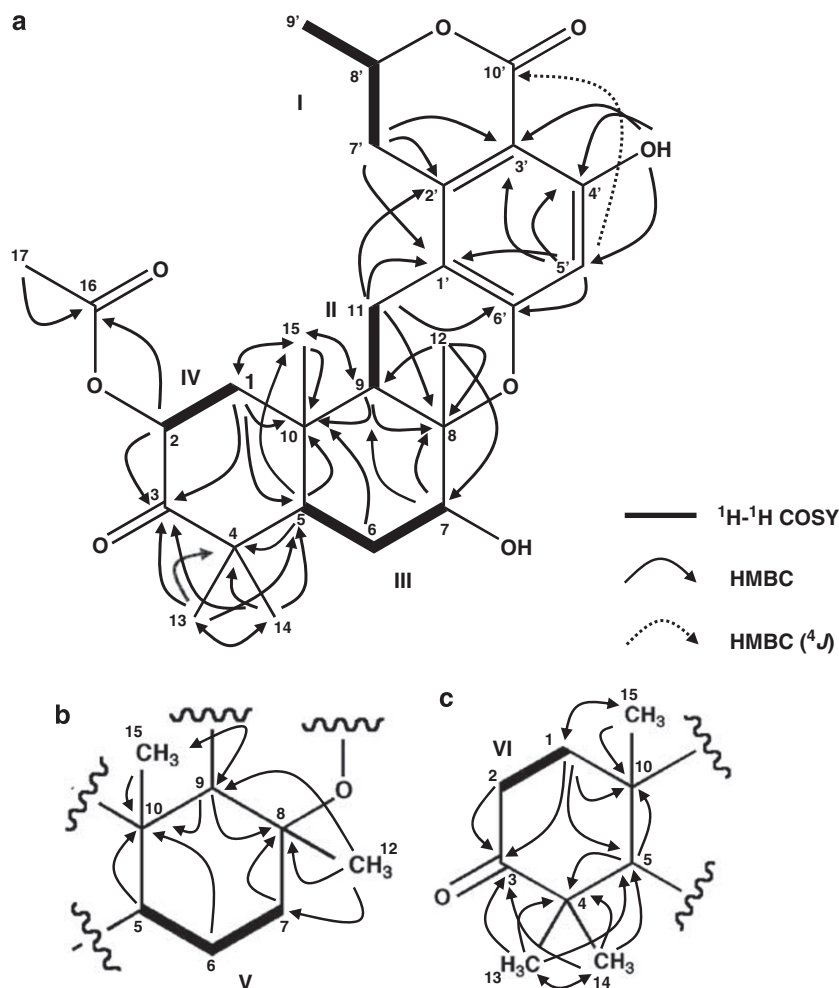


Figure 3 ^1H - ^1H COSY and ^{13}C - ^1H HMBC experiments of pentacecillides A (a), B (b) and C (c).

replaced by methylene protons (δ 2.44, 2.68). Analyses of the ^1H - ^1H COSY revealed that the partial structure VI contains the replaced part (Figure 3c). The partial structure VI was also confirmed by observing cross-peaks from H_3 -15 (δ 0.96) to C-1 (δ 31.7) and C-10 (δ 35.4), from H-5 (δ 1.88) to C-4 (δ 47.2) and C-10, from H_2 -1 (δ 1.67, 2.06) to C-3 (δ 219.6), C-5 (δ 48.1), C-10 and C-15 (δ 23.0), from H_3 -13 (δ 1.09) to C-3, C-4, C-5 and C-14 (δ 29.3), from H_3 -14 (δ 1.12) to C-3, C-4, C-5 and C-13 (20.0) and from H_2 -2 to C-3 in ^{13}C - ^1H HMBC experiments (Figure 3c). Taken together, the structure of pentacecillide A was elucidated as 2-deacetoxy pentacecillide B (Figure 1).

Stereochemistry of pentacecillides

Pentacecillide C has seven chiral carbons in its structure. The relative stereochemistry at C-2, C-5, C-7, C-8, C-9 and C-10 of the tricyclic skeleton (A–B–C) was investigated by NOESY experiments. As shown in Figure 4, cross-peaks were observed between H-2 (δ 5.63) and H_3 -13 (δ 1.20)/ H_3 -15 (δ 1.54), between H_3 -15 and H_{ax} -6 (δ 2.18)/ H_3 -13, between H-5 (δ 1.82) and H-7 (δ 4.11)/H-9 (δ 2.23) and H-7 and H-9, indicating that they are oriented in a 1,3-diaxial conformation. Accordingly, rings A and B are oriented in a chair-chair form. Secondly, cross-peaks were observed between H_3 -12 (δ 1.23) and H-7/ H_2 -11 (δ 2.52) and between H_3 -15 and H_2 -11, suggesting that rings B and C are oriented in a chair-boat form. Thirdly, cross-peaks were observed between H_{eq} -7' (δ 2.85) and H-11/ H -8' (δ 4.62) and

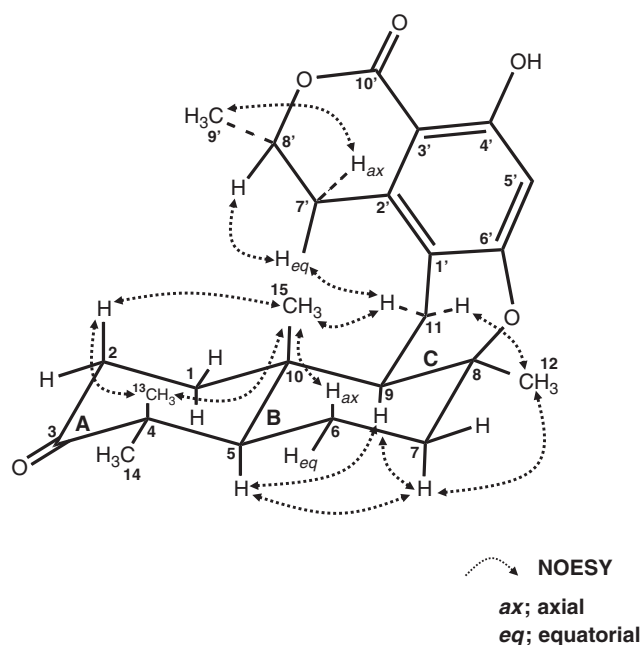


Figure 4 NOESY experiments of pentacecillide C.

between $H_{ax-7'}$ and H_{3-9} (δ 1.55), indicating that the conformation of $H_{3-9'}$ is equatorial, which was also supported by a large coupling constant ($J=11$ Hz) between $H_{ax-7'}$ and $H_{8'}$. Taken together, the relative stereochemistry of pentacecicide C was determined as shown in Figure 1. These results were consistent with those of thailandolide A except for the stereochemistry at C-8', although chemical shifts at C-8' of pentacecicide C and thailandolide A were almost the same.²

The relative stereochemistry of pentacecicides A and B was deduced to be the same as that of pentacecicide C by the similarity of NOESY experiments and the coupling constants in 1H NMR.

DISCUSSION

Pentacecicides A to C, structurally related to thailandolides, were isolated from the culture broth of *P. cecidicola* FKI-3765-1, and were found to have a common pentacyclic core containing an aromatic ring and a δ -lactone ring. The core seemed to be a meroterpene, consisting of a sesquiterpene and a pentaketide. Thailandolides were reported to be produced by *Talaromyces thailandiasis*,² whereas pentacecicides were produced by a different genus *Penicillium*. Thailandolides A and B were not detected in the culture broth of *P. cecidicola* FKI-3765-1.

From the structure elucidation, the planar structures of pentacecicides were seen to be similar to those of thailandolides. The relative stereochemistry of the compounds is almost the same, but the C-8' stereochemistry is different; the C-8' methyl group of thailandolides is

oriented in the axial conformation,² whereas that of pentacecicides is in the equatorial conformation.

METHODS

General experimental procedures

UV spectra were recorded on a spectrophotometer (8453 UV-Visible spectrophotometer, Agilent Technologies Inc., Santa Clara, CA, USA). IR spectra were recorded on a Fourier transform infrared spectrometer (FT-710, Horiba Ltd, Kyoto, Japan). Optical rotations were measured with a digital polarimeter (DIP-1000, JASCO Corporation, Tokyo, Japan). ESI-TOF-MS and HRESI-TOF-MS spectra were recorded on a mass spectrometer (JMS-T100LP, JEOL Ltd, Tokyo, Japan). Various NMR spectra were measured with a spectrometer (XL-400, Varian Inc., Palo Alto, CA, USA).

ACKNOWLEDGEMENTS

This study was supported by the Program for the Promotion of Fundamental Studies in Health Sciences (to HT) from the National Institute of Biomedical Innovation (NIBIO). We express our thanks to Ms N Sato for measuring NMR experiments, and Mr K Nagai and Ms A Nakagawa for measuring mass spectra. We also thank Mr N Ugaki for excellent technical assistance.

1 Yamazaki, H *et al*. Pentacecicides, new inhibitors for lipid droplet formation in mouse macrophages produced by *Penicillium cecidicola* FKI-3765-1. I. Taxonomy, fermentation, isolation and biological properties. *J. Antibiot.* **62**, 195–200 (2009).

2 Dethoup, T *et al*. Merodrimanes and other constituents from *Talaromyces thailandiasis*. *J. Nat. Prod.* **70**, 1200–1202 (2007).