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New class azaphilone produced by a marine fish-derived *Chaetomium globosum*. The stereochemistry and biological activities

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ABSTRACT

Four new metabolites, chaetomugilins P–R and 11-epi-chaetomugilin I, were isolated from a strain of *Chaetomium globosum* originally obtained from the marine fish *Mugil cephalus*, and their absolute stereostructures were elucidated on the basis of spectroscopic analyses, including 1D and 2D NMR techniques and various chemical transformations. Particularly, the skeleton of chaetomugilin P is different from that of other azaphilones isolated from this fungal strain to date. In addition, these compounds significantly inhibited the growth of cultured P388, HL-60, L1210 and KB cell lines.

1. Introduction

Marine products include a number of compounds with unique structures, some of which may exhibit unusual bioactivities. We have focused on potential new antitumor materials from marine-derived microorganisms and found a number of antitumour and/or cytotoxic compounds.[1–3] We have already reported the absolute stereostructures and cytotoxic activities of chaetomugilins A–O, azaphilones isolated as cytostatic metabolites from the fungus *Chaetomium globosum* OUPS-T106B-6 originally obtained from the marine fish *Mugil cephalus*.[4–9] Our continuing search for cytotoxic metabolites from this fungal strain led to the isolation of four new azaphilones designated as chaetomugilins P–R (**1**–**3**) and 11-epi-chaetomugilin I (**4**). Azaphilones have various bioactivities such as antimicrobial activity, nitric oxide inhibition (cohaerins),[10] gp120-CD4 binding inhibition (isochromophiliones, ochrephilone, screotiorin and rubrorotiorin),[11] monoamine oxidase inhibition (luteusins, TL-1, TL-2 and chaetoviridins),[12,13] platelet-derived growth factor binding inhibition (RP-1551s)[14] and antimalarial activity (cochliodones).[15] These metabolites have a chlorine atom at C-5 and a methyl group at C-7, however, chaetomugiline P (**1**) has a methyl group at C-5 and no substituent at C-7. Compounds **1** and **4** exhibited significant cytotoxic activity against the murine P388 leukemia cell line, the human HL-60 leukemia cell line, the murine L1210 leukemia cell line, and the human KB epidermoid carcinoma cell line. We describe herein the absolute configuration of the stereogenic centers and biological activities of these compounds.

2. Results and discussion

2.1. Identification and structure determination

The microorganism from *M. cephalus* fish was cultured at 27 °C for six weeks in a medium (50 L) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4 as reported previously.[4–9] The EtOAc extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicity against the P388 cell line) employing Sephadex LH-20, silica gel column chromatography and the reverse phased HPLC to afford chaetomugilins P–R (**1**–**3**) and 11-epi-chaetomugilin I (**4**) (Fig. 1).

Chaetomugilin P (**1**) was assigned the molecular formula $C_{22}H_{27}ClO_5$ based on the $[M+H]^+$ peak in HRFABMS and the ratio of the intensity of isotope peaks(MH^+/$[MH+2]^+$). Its IR spectrum exhibited bands at 3440, 1678 and 1666 cm^{-1}, characteristic of hydroxyl and α, β-unsaturated carbonyl groups. A close inspection of the ^1H and ^{13}C NMR spectra (Table 1) of **1** in DEPT and HMQC experiments revealed the presence of two secondary methyls (11-CH_3 and C-13), one tertiary methyl (1′-CH_3), one secondary olefin methyl (C-5′), one tertiary olefin methyl (5-CH_3), one sp^3-hybridized methylene (C-7), three sp^3-methines (C-8, C-11 and C-12) including an oxygen-bearing carbon (C-12), five sp^2-methines (C-1, C-4, C-9, C-10 and C-4′) including an oxygen-bearing carbon

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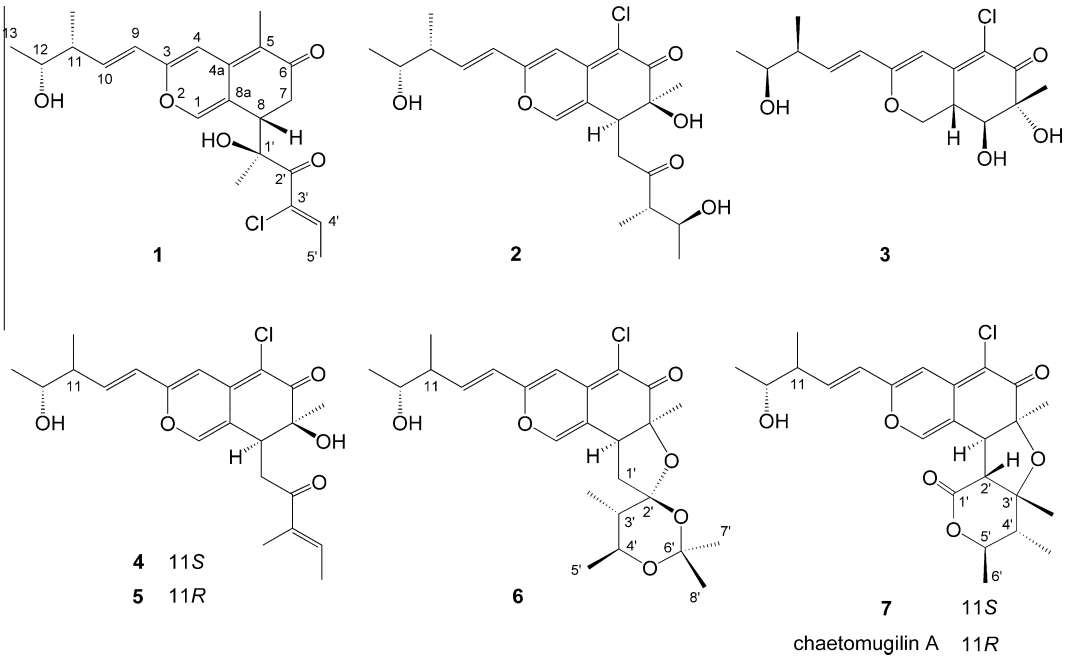


Figure 1. The structures of metabolites from *A. fumigatus* and the derived compound.

Table 1
NMR spectroscopic data ($CDCl_3$) for chaetomugilin P (**1**)

Position	δ_H[a]		J/Hz	NOE	δ_c		HMBC (C)[b]
1	6.71	s		8, 4′, 1′-CH_3, 1′-OH	142.3	(d)	3, 4a, 8, 8a
3					153.2	(s)	
4	5.98	s		5-CH_3	105.5	(d)	3, 8a, 9
4a					139.7	(s)	
5					116.7	(s)	
6					194.6	(s)	
7 A	2.65	dd	16.1 (7B), 7.0 (8)	7B, 8, 1′-CH_3	35.9	(t)	5, 6, 8, 8a
7 B	2.83	dd	16.1 (7A), 2.1 (8)	7A, 8, 1′-CH_3			5, 6, 8, 8a
8	3.44	dd	7.0 (7A), 2.1 (7B)	1, 7A, 7B, 4′, 1′-CH_3	42.0	(d)	1, 4a, 6, 7, 8a, 1′
8a					116.4	(s)	
9	5.98	d	15.6 (10)	10, 11, 13, 11-CH_3	122.9	(d)	3, 4, 10, 11
10	6.32	dd	15.6 (9), 7.8 (11)	9, 11, 12, 13, 11-CH_3	138.3	(d)	3, 11, 12, 11-CH_3
11	2.38	dqd	7.8 (10), 6.9 (11-CH_3), 6.0 (12)	9, 10, 12, 13, 11-CH_3	44.0	(d)	9, 10, 12, 13, 11-CH_3
12	3.77	Quint	6.0 (11,13)	10, 11, 13, 11-CH_3	71.0	(d)	10, 11-CH_3
13	1.17	d	6.0 (12)	9, 10, 11, 12, 11-CH_3	20.3	(q)	11, 12
5-CH_3	1.76	s		4	9.3	(q)	4a, 5, 6
11-CH_3	1.10	d	6.9 (11)	9, 10, 11, 12, 13	14.9	(q)	10, 11, 12
1′					83.8	(s)	
2′					197.2	(s)	
3′					131.1	(s)	
4′	7.38	q	6.9 (5′)	1, 8, 5′, 1′-CH_3, 1′-OH	141.0	(d)	2′, 3′, 5′
5′	2.00	d	6.9 (4′)	4′	15.7	(q)	3′, 4′
1′-CH_3	1.56	s		1, 7A, 7B, 8, 4′	23.8	(q)	8, 1′, 2′
1′-OH	3.33	s		1, 4′			8, 1′, 2′, 1′-CH_3

[a] ^1H chemical shift value (δ ppm) followed by multiplicity and then the coupling constants (J/Hz). Figures in parentheses indicate the proton coupling with that position.
[b] HMBC correlations are from proton stated to the indicated carbon.

(C-1), one quarternary oxygen-bearing sp^3-carbon (C-1′), five quarternary sp^2-carbons (C-3, C-4a, C-5, C-8a and C-3′) including an oxygen-bearing carbon (C-3), and two carbonyls (C-6 and C-2′). The ^1H–^1H COSY analysis of **1** led to three partial structural units as shown by bold-faced lines in Figure 2. The geometrical configuration of the double bond moiety (C-9–C-10) was deduced as the *E* configuration from the coupling constant of the olefinic protons ($J_{9,10}$ = 15.6 Hz). The connection of these units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 2, and this fact suggested that a chlorine atom connected to C-3′, a quarternary olefin carbon. In addition, the geometry for the double bond moiety (C-3′–C-4′) was deduced as the *Z* configuration from NOE correlations (H-4′/H-8 and H-4′/1′-CH_3). Thus the planar structure of **1** was elucidated as shown in Figure 2. To determine the absolute configurations for C-8, C-11, C-12 and C-1′, **1** was transformed to chaetomugilin I (**5**), whose absolute stereostructure had been determined.[8] The treatment of **1** in MeOH with *p*-TsOH gave the product **5** (yield 21.4%) (Scheme 1), which was confirmed to be identical to the natural substance **5** in IR, UV, NMR spectra and optical rotation. As shown in Scheme 1, the plausible mechanism for this reaction is unusual, with both Diels-Alder and retro-Diels–Alder reactions occuring.

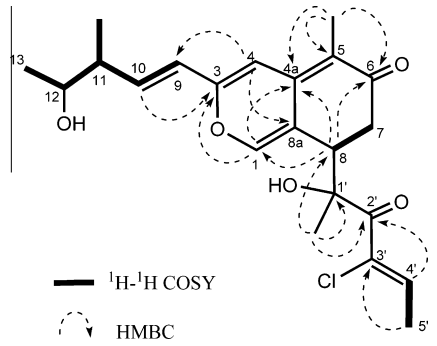
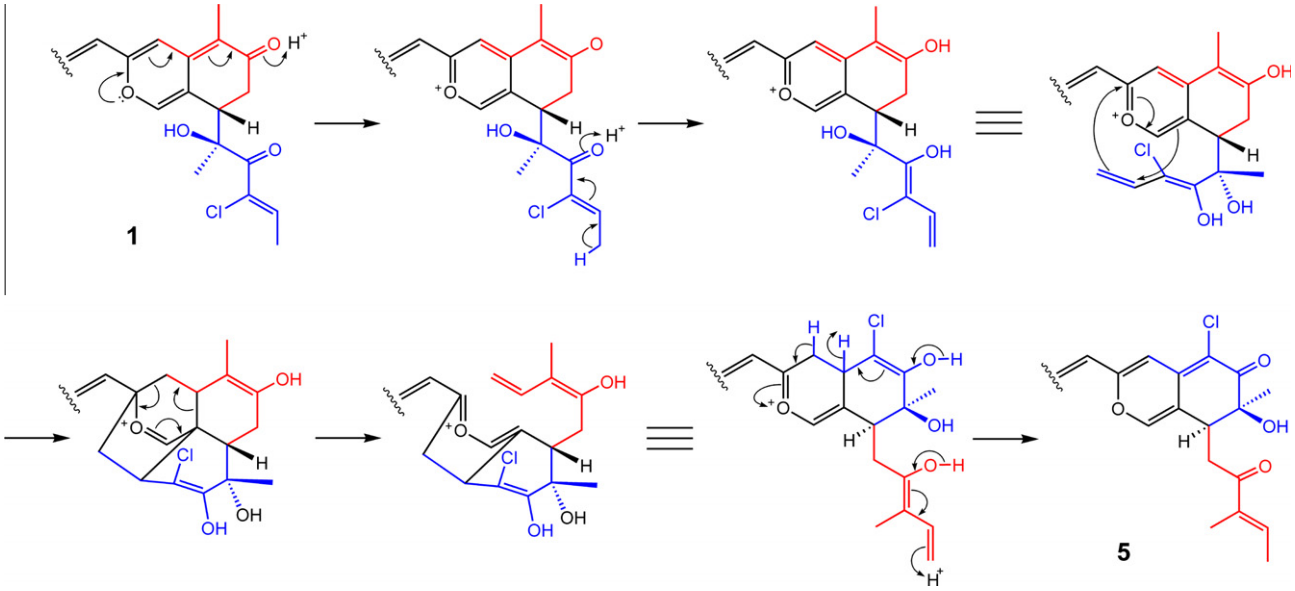


Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations in **1**.

This conversion from **1** to **5** revealed the absolute stereostructure of **1** (8*S*, 11*R*, 12*R* and 1′*S*). The CD spectra of **1** and **5** exhibited typical exciton spilt Cotton effects, each sign of which agreed with that of the screw sense between the side chain enone chromophore (C-2′ and C-3′) and the conjugated chromophore (C-4–C-6) of the azaphilone moiety (Fig. 3).

Chaetomugilin Q (**2**) was assigned the molecular formula $C_{22}H_{29}ClO_6$ deduced from HRFABMS. Its general spectral features closely resembled those of **5** except for ^{1}H and ^{13}C NMR signals at C-2′–C-5′ (Table 2). The planar structure of **2** was confirmed by analyzing ^{1}H–1H COSY and HMBC correlations (from 3′-CH_3 to C-2′, from H-4′ to C-2′, and from H-5′ to C-3′). The above evidence and the ^{13}C NMR chemical shift of C-4′ (δ_C 69.74), together with the molecular formula of **2**, suggested the addition of H_2O to the double bond (C-3′–C-4′) in **5**. The absolute configurations at C-7, C-8, C-11 and C-12 in **2** were determined by the transformation from **2** to **5**. The treatment of **2** in MeOH with *p*-TsOH produced **5** with the elimination of H_2O (yield 20.9%), which was confirmed to be identical with the natural **5** in all spectral data and optical rotation.



Scheme 1. Plausible mechanism for transformation from **1** to **5**.

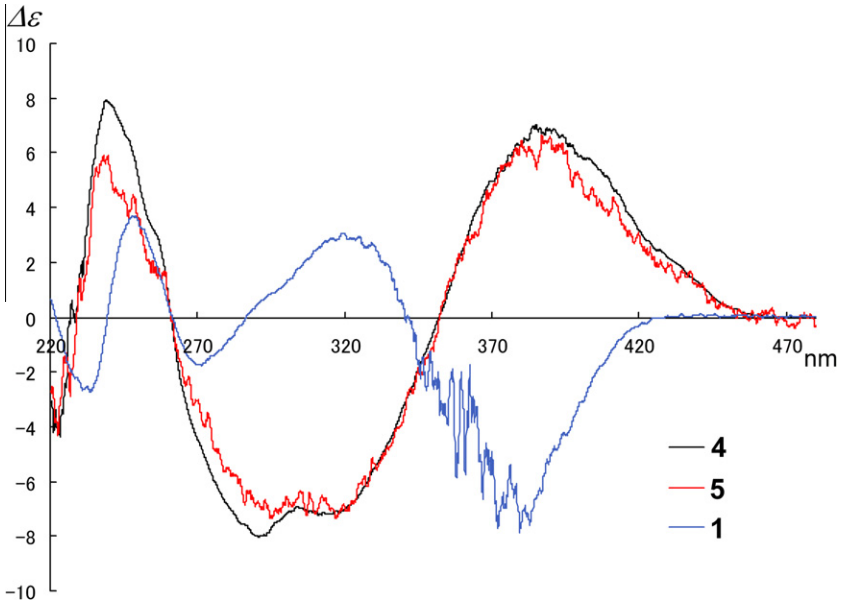


Figure 3. CD spectra of **1**, **4** and **5**.

Table 2
NMR spectroscopic data ($CDCl_3$) for chaetomugilin Q (**2**) and acetonide **6**

	Chaetomugilin Q (**2**)					Acetinide **6**		
Position	δ_H[a] (J/Hz)		δ_C, multi.		HMBC (C)[b]	δ_H[a] (J/Hz)		δ_c
1	7.42	s	145.2	(d)	3, 4a, 8, 8a	7.21	s	143.5 (d)
3			156.7	(s)				156.3 (s)
4	6.47	s	104.9	(d)	3, 5, 8a, 9	6.48	s	105.1 (d)
4a			141.4	(s)				139.2 (s)
5			107.0	(s)				110.9 (s)
6			191.7	(s)				188.8 (s)
7			74.1	(s)				85.1 (s)
8	3.46	dd (9.5, 3.5)	40.6	(d)	1, 4a, 6, 7, 8a, 7-CH_3, 1′, 2′	3.45	dd (12.5, 7.2)	42.7 (d)
8a			119.5	(s)				116.6 (s)
9	6.11	d (15.5)	122.2	(d)	3, 4, 10, 11	6.12	d (15.5)	122.4 (d)
10	6.59	dd (15.5, 8.0)	142.0	(d)	3, 11, 12, 11-CH_3	6.57	dd (15.5, 8.0)	141.2 (d)
11	2.44	dqd (8.0, 6.5, 5.8)	44.1	(d)	9, 10, 12, 11-CH_3	2.44	dqd (8.0, 6.5, 6.0)	44.1 (d)
12	3.81	qd (6.5, 5.8)	70.8	(d)	10, 11, 13, 11-CH_3	3.81	Quint (6.0)	70.9 (d)
13	1.19	d (6.5)	20.4	(q)	11, 12	1.19	d (6.0)	20.4 (q)
7-CH_3	1.31	s	26.6	(q)	6, 7, 8	1.44	s	25.0 (q)
7-OH	4.03	br s			6, 7, 8			
11-CH_3	1.12	d (6.5)	14.7	(q)	10, 11, 12	1.13	d (6.5)	14.8 (q)
1′ A	2.39	dd (18.0, 9.5)	40.9	(t)	7, 8, 8a, 2′	2.20	t (12.5)	42.7 (t)
1′ B	3.2	dd (18.0, 3.5)			7, 8, 8a, 2′	1.85	dd (12.5, 7.2)	
2′			213.7	(s)				106.4 (s)
3′	2.41	dq (7.5, 7.0)	54.0	(d)	2′, 4′, 5′, 3′-CH_3	1.56	dq (10.5, 7.0)	40.8 (d)
4′	3.86	dq (7.5, 6.1)	69.7	(d)	2′, 3′, 5′, 3′-CH_3	4.09	dq (10.5, 6.0)	67.3 (d)
5′	1.13	d (6.1)	20.9	(q)	3′, 4′	1.17	d (6.0)	19.9 (q)
3′-CH_3	1.04	d (7.0)	13.7	(q)	2′, 3′, 4′	0.75	d (7.0)	11.3 (q)
6′								98.8 (s)
7′						1.63	s	25.0 (q)
8′						1.35	s	31.2 (q)

[a] As in Table 1.
[b] As in Table 1.

The above findings revealed the absolute configuration of the chiral centers as 7*S*, 8*S*, 11*R* and 12*R* except for C-3′ and C-4′. To determine the absolute configurations of C-3′ and C-4′ in **2**, we designed an acetonide, in which an isopropyridene group is interposed between 7-OH and 4′-OH. The treatment of **2** in $CHCl_3$ with 2, 2-dimethoxypropane gave the acetonide **6** (yield 33.1%), however, the ^{13}C NMR data for **6** suggested that the carbonyl group at C-2′ (δ_C 213.7) in **2** was replaced by an acetal (δ_C 106.4) in **6** (Table 2). HMBC correlations (from H-4′ to C-6′) and HRFABMS ($[M+H]^+$ *m/z* 465.2020) in **6** revealed that ether linkages were formed between C-2′and C-7, and an acetonide was bridged between C-2′and C-4′. In the NOESY experiment of **6**, observed NOEs (H-1′/3′-CH_3, 3′-CH_3/7-CH_3 and H-4′/H-7′) implied that 3′-CH_3 is oriented *trans* to 4′-CH_3, and the C-2′ – C-3′ bond is *cis* to 7-CH_3 (Fig. 4). In addition, NOE correlation from H-7′ to H-4 and *J* value ($J_{3',4'}$ = 10.5 Hz) implied the six members ring including an isopropyridene group to exist in a chair conformation. These evidences revealed the absolute configuration for C-3′ and C-4′ in **2** to be *S* and *S*, respectively.

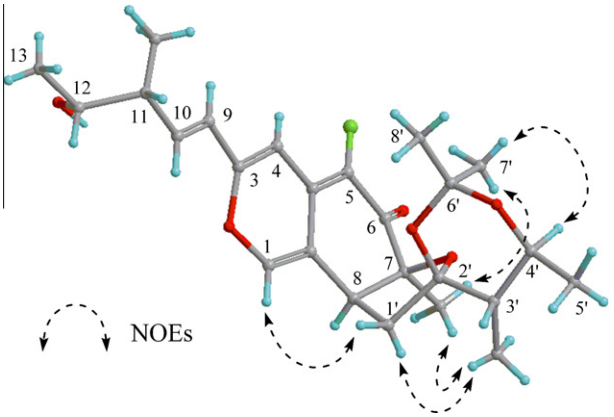


Figure 4. Key NOEs for **6**.

Chaetomugilin R (**3**) was assigned the molecular formula $C_{16}H_{21}ClO_5$ which contained six carbons atom less than that of **5**. In the ^1H and ^{13}C NMR spectra (Table 3), the signals for C-1′–C-5′ in **5** disappeared, and a hydroxyl methine (δ_H 3.73, δ_C 73.89), a methylene (δ_H 3.81, 4.84, δ_C 68.43) and a sp^3-methine (δ_H 2.88, δ_C 38.27) newly appeared in **3**. The geometrical configuration of the double bond moiety (C-9–C-10) was deduced as *trans* from the coupling constant of the olefinic protons ($J_{9,10}$ = 15.5 Hz). The ^1H–^1H COSY correlations (H-1/H-8a and H-8a/H-8) and the HMBC correlations (from 7-CH_3 to C-8, H-1 to C-3, H-4 to C-8a, and H-8a to C-5) revealed the planar structure of **3**. In NOESY experiments for **3**, the NOE correlations (H-8a/7-CH_3 H-8a/H-1β and H-1α/H-8) implied that 7′-CH_3 is oriented *trans* to H-8, and *cis* to H-8a (Fig. 5). In order to determine the absolute configurations at C-11 and C-12 in **3**, the alkaline degradation of **3** was carried out.[8] The degradation of **3** with 5% potassium hydroxide afforded a carboxylic acid, which was identified with 2*E*-5-hydroxy-4-methylhex-2-enoic acid obtained from **5** by a similar manner in IR, UV and NMR spectra except the specific rotations. The carboxylic acid obtained from **3** showed the $[\alpha]_D$ value opposite that obtained from **5**. This evidence revealed that the absolute configurations for C-11 and C-12 of **3** were established as *S* and *S*, respectively. In addition, the modified Mosher's method[16] was used to determine the absolute configuration of C-8 in **3**, and the ^1H chemical-shift difference between (*R*)- and (*S*)-MTPA esters (**3a** and **3b**) implied an *S* configuration at C-8a, and supported an *S* configuration at C-12 (Fig. 6). These evidences allowed assignment of the absolute configuration of all the asymmetric centers (7*R*, 8*S*, 8a*S*, 11*S* and 12*S*).

11-Epi-chaetomugilin I (**4**) had the same molecular formula ($C_{22}H_{27}ClO_5$) as **1** and **5** as deduced from HRFABMS. The general features of its UV, IR and NMR spectra (Table 4) closely resembled those of chaetomugilin I (**5**) except that the NMR signals for the

Table 3
NMR spectroscopic data ($CDCl_3$) for chaetomugilin R (**3**)

Position	δ_H[a]		J/Hz	NOE	δ_c		HMBC (C)[b]
1 α	3.81	dd	13.1 (8a), 11.0 (1β)	1β, 8	68.4	(t)	3, 4a, 8a
1 β	4.84	dd	11.0 (1α), 5.0 (8a)	1α, 8a			3, 4a, 8a
3					162.4	(s)	
4	6.02	s			100.9	(d)	3, 5, 8a, 9
4a					146.6	(s)	
5					117.5	(s)	
6					193.2	(s)	
7					77.9	(s)	
8	3.73	d	10.0 (8a)	1α	73.9	(d)	1, 7, 8a, 7-CH_3
8a	2.88	ddd	13.1 (1α), 10.0 (8), 5.0 (1β)	1β, 7-CH_3	38.3	(d)	1, 4a, 5, 8
9	6.04	d	15.5 (10)	10, 11, 11-CH_3	124.6	(d)	3, 4, 10, 11
10	6.55	dd	15.5 (9), 7.5 (11)	9, 11, 12, 13, 11-CH_3	142.0	(d)	3, 11, 12, 11-CH_3
11	2.42	dqd	7.5 (10), 6.3 (11-CH_3), 6.0 (12)	9, 10, 12, 13, 11-CH_3	44.2	(d)	9, 10, 12, 13, 11-CH_3
12	3.79	Quint	6.0 (11, 13)	10, 11, 13, 11-CH_3	71.0	(d)	10, 11-CH_3
13	1.19	d	6.0 (12)	10, 11, 12	20.4	(q)	11, 12
7-CH_3	1.34	s		8a	19.1	(q)	6, 7, 8
11-CH_3	1.11	d	6.3 (11)	9, 10, 11, 12	14.8	(q)	10, 11, 12

[a] As in Table 1.
[b] As in Table 1.

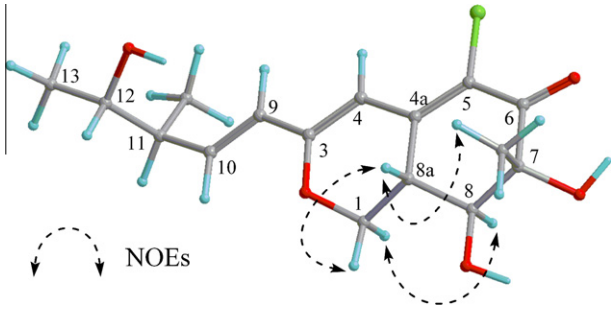


Figure 5. Key NOEs for **3**.

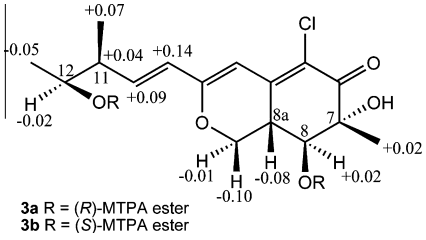


Figure 6. ^1H chemical-shift difference ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**3a** and **3b**).

side chain moiety (C-9–C-13) [the proton signals; H-11 (δ_H 2.35, dqd), H-12 (δ_H 3.74, quint), the carbon signal; 11-CH_3 (δ_C 16.11)] revealed a chemical shift difference. The ^1H–^1H COSY and HMBC correlations (vide info) led to elucidation of the planar structure of **4**. In terms of the stereochemistry, the CD spectra of **4** and **5** showed similar Cotton curves, allowing for the assignment of the absolute configuration of the asymmetric centers, except C-11 and C-12 (7*S* and 8*S*) (Fig. 3). This suggested that **4** was the stereoisomer of **5** at C-11 or C-12. Most chaetomugilins produced by this fungal strain have the *R* configuration at C-11 and *R* configuration at C-12, however, we had isolated 11-epi-chaetomugilin A (**7**), which had the opposite absolute configuration at C-11 to chaetomugilin A.[9] The ^1H and ^{13}C NMR signals for the core part of **7** closely resembled those of chaetomugilin A. In the side chain moiety, the chemical shift difference of the proton signals for H- 11 and H-12 and the carbon signal for 11-CH_3 (Table 4) had been observed. In comparison between **4** and **5** in NMR spectra, the difference was the same as the above feature for **7** and chaetomugilin A. Based on this evidence, we guessed that **4** was a epimer of **5** at C-11. In future, we will carry out the modified Mosher's method to confirm the absolute configuration at C-12.

2.2. Cytotoxic activities

As a primary screen for antitumor activity, cancer cell growth inhibitory properties of chaetomugilins P–R (**1**–**3**) and 11-epi-chaetomugilin I (**4**) were examined using the murine P388 leukemia cell line, the human HL-60 leukemia cell line, the murine L1210 leukemia cell line, and the human KB epidermoid carcinoma cell line. As shown in Table 5, **1** and **4** exhibited significant cytotoxic activity against the cancer cell lines, more than 5-FU used as a positive control. This result suggests that the cytotoxicity requires an enone moiety at C-2′–C-4′, because the activities of **1** and **4** were nearly equal to that of **5**. Henceforth, compounds **1** and **2** will be examined using a disease-oriented panel of 39 human cell lines[7,8] to reveal their selective cytotoxic activity and mode of action.

3. Conclusion

The marine fish-derived fungus *C. globosum* OUPS-T106B-6 produced chaetomugilins P–R (**1**–**3**) and 11-epi-chaetomugilin I (**4**) as metabolites with growth inhibitory effects on cancer cells. Interestingly, chaetomugilin P (**1**) had a unique skeleton and is a new class of azaphilones. **1** and **4** have exhibited significant potent cytotoxicity, and their activities are equal to that of 5-fluorouracil in IC_{50} value.

4. Experimental

4.1. General procedures

Mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. UV spectra were recorded on a Hitachi U-2000 spectrophotometer and IR spectra on a JASCO FT/IR-680 plus. NMR spectra were recorded at 27 °C on Varian UNITY INOVA-500 and MERCURY spectrometers with tetramethylsilane (TMS) as an internal reference. FABMS was determined using a JOEL JMS-700 (Ver. 2) mass spectrometer. Optical rotations were recorded on a JASCO J-820 polarimeter. Liquid chromatography

Table 4
(a) NMR spectroscopic data ($CDCl_3$) for 11-epi-chaetomugilin I (**4**) and chaetomugilin I (**5**). (b) NMR spectroscopic data ($CDCl_3$) for 11-epi-chaetomugilin A (**7**) and chaetomugilin A

11-Epi-chaetomugilin I (4)					Chaetomugilin I (5)			
Position	δ_H[a]		δc		δ_H[a]		δ_c	
1	7.47	s	145.7	(d)	7.48	s	145.7	(d)
3			156.5	(s)			156.7	(s)
4	6.47	s	105.1	(d)	6.47	s	105.0	(d)
4a			141.6	(s)			141.6	(s)
5			106.9	(s)			106.8	(s)
6			191.9	(s)			191.9	(s)
7			74.1	(s)			74.1	(s)
8	3.48	dd	40.5	(d)	3.48	dd	40.5	(d)
8a			119.5	(s)			119.5	(s)
9	6.12	d	122.8	(d)	6.12	d	122.3	(d)
10	6.56	dd	141.8	(d)	6.58	dd	141.9	(d)
11	2.35	dqd	44.2	(d)	2.44	sex	44.2	(d)
12	3.74	Quint	71.1	(d)	3.80	Quint	70.8	(d)
13	1.21	d	21.0,	(q)	1.19	d	20.4	(q)
7-CH_3	1.33	s	26.8,	(q)	1.33	s	26.7	(q)
11-CH_3	1.11	d	16.1	(q)	1.12	d	14.8	(q)
1′ A	2.62	dd	34.8	(t)	2.62	dd	34.8	(t)
1′ B	3.27	dd			3.27	dd		
2′			199.4	(s)			199.4	(s)
3′			138.0	(s)			138.0	(s)
4′	6.66	q	138.0	(d)	6.66	q	138.0	(d)
5′	1.81	d	14.7	(q)	1.81	d	14.7	(q)
3′-CH_3	1.73	s	11.0	(q)	1.73	s	10.5	(q)

11-Epi-chaetomugilin A (**7**)					Chaetomugilin A			
Position	δ_H[a]		δ_c, multi.		δ_H[a]		δ_c	
1	7.29	s	145.5	(d)	7.27	s	145.7	(d)
3			157.1	(s)			157.1	(s)
4	6.57	s	105.4	(d)	6.57	s	105.5	(d)
4a			140.2	(s)			140.1	(s)
5			110.3	(s)			110.3	(s)
6			189.2	(s)			189.3	(s)
7			83.8	(s)			84.0	(s)
8	3.00	d	50.4	(d)	2.98	d	50.6	(d)
8a			114.4	(s)			114.3	(s)
9	6.16	d	122.5	(d)	6.15	d	122.1	(d)
10	6.62	dd	142.5	(d)	6.61	dd	142.5	(d)
11	2.39	sex	44.8	(d)	2.45	sex	44.3	(d)
12	3.77	Quint	71.0	(d)	3.81	br s	70.9	(d)
13	1.22	d	20.9	(q)	1.20	d	20.5	(q)
7-CH_3	1.40	s	23.3	(q)	1.40	s	23.2	(q)
11-CH_3	1.12	d	16.1	(q)	1.13	d	14.8	(q)
1′			170.7	(s)			170.7	(s)
2′	3.05	d	58.2	(d)	3.06	d	58.2	(d)
3′			104.0	(s)			104.2	(s)
4′	1.90	dq	44.9	(d)	1.90	dq	44.9	(d)
5′	4.31	dq	77.2	(d)	4.30	dq	76.9	(d)
6′	1.41	d	18.7	(q)	1.41	d	18.7	(q)
4′-CH_3	1.13	d	8.8	(q)	1.13	d	8.8	(q)

[a] As in Table 1

Table 5
Cytotoxity of the metabolites against P388, HL-60, L1210 and KB cells

Compounds	Cell line P388 IC_{50} (pM)[a]	Cell line HL-60 IC_{50} (pM)[a]	Cell line L1210 IC_{50} (pM)[a]	Cell line KB IC_{50} (pM)[a]
Chaetomugilin I (5)	1.1	1.1	1.9	2.3
P (1)	0.7	1.2	1.5	1.8
Q (2)	49.5	47.2	80.2	>100
R (3)	32.0	51.8	67.1	67.1
11-Epichaetomugilin I (4)	0.7	1.0	1.6	1.2
5-FU[b]	1.7	2.7	1.1	7.7

[a] DMSO was used for vehicle.
[b] Positive control.

over silica gel (mesh 230–400) was performed at medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent system CH_2Cl_2–MeOH (19:1), and compounds were viewed under a UV lamp, and sprayed with 10% H_2SO_4, and then heated.

4.2. Culturing and isolation of metabolites

A strain of *C. globosum* was initially isolated from the marine fish *M. cephalus*, collected in Katsuura bay, Japan in October 2000. The marine fish was wiped with EtOH and its gastrointestinal tract applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *C. globosum*. The fungal strain was cultured at 27 °C for six weeks in a liquid medium (50 L) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4. The culture filtrate was extracted thrice with EtOAc. The combined extracts were evaporated in vacuo to afford a mixture of crude metabolites (20.3 g), which exhibited cytotoxicity (ED_{50} 35.8 μg/ml). The EtOAc extract was passed through Sephadex LH-20, using $CHCl_3$/MeOH (1:1) as the eluent. The second fraction (7.2 g), in which the activity was concentrated, was chromatographed on a silica gel column with a $CHCl_3$–MeOH gradient as the eluent. The MeOH/$CHCl_3$ (1:99) eluate (489.3 mg) was purified by HPLC using MeCN/H_2O (50:50) as the eluent to afford **1** (4.1 mg), **4** (2.9 mg) and **5** (15.7 mg). The MeOH–$CHCl_3$ (1:99) eluate (1.8 g) was purified by HPLC using MeCN/H_2O (35:65) as the eluent, following HPLC using MeCN/H_2O (50:50) as the eluent, to afford **2** (7.4 mg) and **7** (6.2 mg). The MeOH/$CHCl_3$ (2:98) eluate (220.7 mg) was purified by HPLC using MeCN/H_2O (35:65) as the eluent to afford **3** (3.3 mg).

Chaetomugilin P (**1**): yellow powder; mp 103–105 °C ($CHCl_3$–MeOH); $[\alpha]_D^{22}$ −49.7 (*c* 0.06, EtOH); UV λ_{max} (EtOH) nm (log ε) 283 (3.54), 384 (3.68), 415 (3.24); IR ν_{max} (KBr) cm^{-1} 3440, 1678, 1666, 1563, 1522; HRFABMS *m/z*, found 407.1618 $[M+H]^+$ (calcd for $C_{22}H_{28}{}^{35}ClO_5$, 407.1626). ^1H and ^{13}C NMR data are listed in Table 1.

Chaetomugilin Q (**2**): yellow powder; mp 110–112 °C ($CHCl_3$–MeOH); $[\alpha]_D^{22}$ −18.3 (*c* 0.14, EtOH); UV λ_{max} (EtOH) nm (log ε) 291 (3.77), 373 (3.75), 412 (3.83); IR ν_{max} (KBr) cm^{-1} 3407, 1642, 1560, 1522; HRFABMS *m/z*, found 425.1728 $[M+H]^+$ (calcd for $C_{22}H_{30}{}^{35}ClO_6$, 425.1731). ^1H and ^{13}C NMR data are listed in Table 2 and S1 (Supplementary data).

Chaetomugilin R (**3**): yellow powder; mp 104–106 °C ($CHCl_3$–MeOH); $[\alpha]_D^{22}$ −130.2 (*c* 0.11, EtOH); UV λ_{max} (EtOH) nm (log ε) 331 (3.73), 371 (3.73), 393 (3.78); IR ν_{max} (KBr) cm^{-1} 3424, 1666, 1564, 1547; HRFABMS *m/z*, found 329.1149 $[M+H]^+$ (calcd for $C_{16}H_{22}{}^{35}ClO_5$, 329.1156). ^1H and ^{13}C NMR data are listed in Table 3.

11-Epi-chaetomugilin I (**4**) yellow powder; mp 85–87 °C ($CHCl_3$–MeOH); $[\alpha]_D^{22}$ 77.5 (*c* 0.12, EtOH); UV λ_{max} (EtOH) nm (log ε) 290 (3.87), 373 (3.84), 410 (3.84); IR ν_{max} (KBr) cm^{-1} 3448, 1645, 1617, 1560, 1522; HRFABMS *m/z*, found 407.1618 $[M+H]^+$ (calcd for $C_{22}H_{28}{}^{35}ClO_5$: 391.1626). ^1H and ^{13}C NMR data are listed in Table 4 and S3 (Supplementary data).

4.3. Transformation of 1 and 2–5

p-TsOH (0.5 mg) was added to a MeOH solution (1 mL) of chaetomugilin P (**1**) (5.6 mg), and the reaction mixture was left at room temperature for 3 h. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeCN/H_2O (50:50) as the eluent to afford **5** (1.2 mg).[8]

Using the same proceudure as above with **1**, chaetomugilin Q (**2**) (10.5 mg) was treated with *p*-TsOH (1.9 mg) in MeOH (1 mL)

and the products were purified by HPLC using MeCN/H_2O (50:50) as the eluent to afford **5** (2.1 mg).[8]

4.4. Formation of the acetonide 6 from 2

2,2-Dimethoxypropane (1 mL) and *p*-toluenesulfonate (3.6 mg) were added to a CH_2Cl_2 solution (1 mL) of chaetomugilin Q (**2**) (17.9 mg), and the reaction mixture was left at room temperature for 2 h. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeCN/H_2O (70:30) as the eluent to afford the acetonide **6** (6.5 mg) as a yellow powder.

Acetonide **6**: $[\alpha]_D^{22}$ −105.0 (*c* 0.16, EtOH); FABMS *m/z* (rel. int.) 465 ([M+H]$^+$, 100%); HRFABMS *m/z* found 465.2050 [M+H]$^+$ (calcd for $C_{25}H_{34}{}^{35}ClO_6$, 465.2054). ^{1}H and ^{13}C NMR data are listed in Table 2 and S2 (Supplementary data).

4.5. Degradation by potassium hydroxide of 3

Chaetomugilin R (**3**) (18.6 mg) was dissolved in 10 mL of 5% aq potassium hydroxide and stirred for 3 h at 100 °C. The reaction mixture was extracted with 10 mL of $CHCl_3$. The water layer was adjusted to pH 3.0 with 9% sulfuric acid and reextracted with 10 mL of AcOEt. The organic extract was concentrated to dryness in vacuo. The residue was purified by HPLC using a MeCN/H_2O gradient (0:100)–(60:40) as the eluent to afford (4*S*, 5*S*)-2*E*-5-hydroxy-4-methylhex-2-enoic acid (0.6 mg) as a colorless oil. Using the same procedure, chaetomugilin I (**5**) (16.8 mg), whose absolute stereostructure had been established, was treated with 5% aq potassium hydroxide (10 mL), and purified by HPLC to afford (4*R*, 5*R*)-2*E*-5-hydroxy-4-methylhex-2-enoic acid (0.5 mg) as a colorless oil.

(4*R*, 5*R*)-2*E*-5-hydroxy-4-methylhex-2-enoic acid: $[\alpha]_D^{22}$ 90.0 (*c* 0.05, EtOH); HRFABMS *m/z:* 145.0867 found [M+H]$^+$ (calcd for $C_7H_{13}O_3$,145.0865); ^{1}H NMR δ ppm ($CDCl_3$): 1.12 (3H, d, *J* = 6.5 Hz, 4-CH_3), 1.19 (3H, d, *J* = 6.2 Hz, H-6), 2.44 (1H, dqd, *J* = 7.5, 6.5, 6.2 Hz, H-4), 3.80 (1H, quint, *J* = 6.2 Hz, H-5), 5.90 (1H, d, *J* = 15.5 Hz, H-2), 7.06 (1H, dd, *J* = 15.5, 7.5 Hz, H-3).

(4*S*, 5*S*)-2*E*-5-hydroxy-4-methylhex-2-enoic acid: $[\alpha]_D^{22}$ –89.8 (*c* 0.06, EtOH); HRFABMS *m/z:* 145.0868 found [M+H]$^+$ (calcd for $C_7H_{13}O_3$, 145.0865).

4.6. Formation of the (*R*)- and (*S*)-MTPA esters 3a and 3b from 3

(*R*)-MTPA (4.0 mg), dicyclohexylcarbodiimide (DCC) (4.0 mg) and 4-(dimethylamino) pyridine (DMAP) (1.0 mg) were added to a CH_2Cl_2 solution (0.5 mL) of chaetomugilin R (**3**) (2.0 mg), and the reaction mixture was left at room temperature for 3 h. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC using MeCN/H_2O (85:15) as the eluent to afford (*R*)-MTPA ester **3a** (1.8 mg) as a yellow powder. The same reaction with **3** (2.3 mg) using (*S*)-MTPA (4.8 mg) gave the ester **3b** (1.7 mg).

Compuond **3a**: FABMS *m/z* (rel. int.) 761 ([M+H]$^+$, 23.3%). HRFABMS *m/z* found 761.1943 [M+H]$^+$ (calcd for $C_{36}H_{36}{}^{35}ClF_6O_9$, 761.1952); ^{1}H NMR δ ppm ($CDCl_3$): 1.03 (3H, d, *J* = 6.8 Hz, 11-CH_3), 1.31 (3H, d, *J* = 6.5 Hz, H-13), 1.33 (3H, s, 7-CH_3), 2.57 (1H, dqd, *J* = 8.0, 6.8, 6.5 Hz, H-11), 3.10 (1H, ddd, *J* = 13.1, 12.0, 5.0 Hz, H-8a), 3.55 (3H, s, MTPA–OCH_3), 3.57 (3H, s, MTPA–OCH_3), 3.87 (1H, dd, *J* = 13.1, 11.0 Hz, H-1′α), 4.38 (1H, dd, *J* = 11.0, 5.0 Hz, H-1′β), 5.13 (1H, quint, *J* = 6.5 Hz, H-12), 5.35 (1H, d, *J* = 12.0 Hz, H-8), 5.81 (1H, d, *J* = 15.5 Hz, H-9), 6.00 (1H, s, H-4), 6.32 (1H, dd, *J* = 15.5, 8.0 Hz, H-10), 7.38-7.43 (3H, m, Ar–H), 7.43–7.47 (3H, m, Ar–H), 7.52–7.56 (2H, m, Ar–H), 7.60–7.64 (2H, m, Ar–H).

Compuond **3b**: FABMS *m/z* (rel. int.): 761 ([M+H]$^+$, 14.9%). HRFABMS *m/z* found 761.1940 [M+H]$^+$ (calcd for $C_{36}H_{36}{}^{35}ClF_6O_9$, 761.1952); ^{1}H NMR δ ppm ($CDCl_3$): 1.10 (3H, d, *J* = 6.5 Hz, 11-CH_3), 1.26 (3H, d, *J* = 6.0 Hz, H-13), 1.35 (3H, s, 7-CH_3), 2.61 (1H, dqd, *J* = 7.5, 6.5, 6.0 Hz, H-11), 3.02 (1H, ddd, *J* = 13.1, 11.5, 5.0 Hz, H-8a), 3.51 (3H, s, MTPA–OCH_3), 3.59 (3H, s, MTPA–OCH_3), 3.86 (1H, dd, *J* = 13.1, 11.0 Hz, H-1′α), 4.28 (1H, dd, *J* = 11.0, 5.0 Hz, H-1′β), 5.11 (1H, quint, *J* = 6.0 Hz, H-12), 5.37 (1H, d, *J* = 11.5 Hz, H-8), 5.95 (1H, d, *J* = 15.5 Hz, H-9), 6.00 (1H, s, H-4), 6.48 (1H, dd, *J* = 15.5, 7.5 Hz, H-10), 7.37–7.42 (3H, m, Ar–H), 7.42–7.47 (3H, m, Ar–H), 7.49–7.52 (2H, m, Ar–H), 7.67–7.70 (2H, m, Ar–H).

4.7. Cytotoxic assay

Cytotoxic activities of chaetomugilins P–R (**1**–**3**) and 11-epi-chaetomugilin I (**4**) were examined by the 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. P388, HL-60, L1210 and KB cells were cultured in the Eagle's Minimum Essential Medium (10% fetal carf serum) at 37 °C in 5% CO_2. The test material was dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 10 mm, and the solution was diluted with the Essential Medium to give concentrations of 200, 20 and 2 μM, respectively. Each solution was combined with each cell suspension (1×10^5 cells/ml) in the medium, respectively. After incubation at 37 °C for 72 h in 5% CO_2, the grown cells were labeled with 5 mg/ml MTT in phosphate-buffered saline (PBS), and the absorbance of formazan dissolved with 20% sodium dodecyl sulfate (SDS) in 0.1 N HCl was measured at 540 nm using microplate reader (Model 450, BIO-RAD). Each value was expressed as a percentage, relative to a control cell suspension prepared without the test substance. All assays were performed three times, semilogarithmic plots were constructed from the averaged data, and the effective dose of the substance required to inhibit cell growth by 50% (IC_{50}) was determined.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.008.

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