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Two anti-inflammatory chlorinated azaphilones from *Chaetomium globosum* TW1-1 cultured with 1-methyl-L-tryptophan and structure revision of chaephilone C

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ABSTRACT

By feeding 1-methyl-L-tryptophan (1-MT) into the inactivated rice cultures of an arthropod-derived fungus *Chaetomium globosum* TW1-1, two new chlorinated azaphilones (**1** and **2**) were isolated and identified. Their structures incorporating absolute configurations were determined by comprehensive spectroscopic analyses and single-crystal X-ray diffraction. Remarkably, chaephilone C, which was reported to possess an unprecedented 6-6-5-6-fused ring system with a chlorine substitution, was structurally revised to **1**. Chaephilones C (**1**) and D (**2**) showed moderate anti-inflammatory activity against the NO production with IC_{50} values of 15.12 ± 1.48 and 20.65 ± 0.95 μM, respectively.

As a well-known filamentous fungus of the *Chaetomiaceae* family, *Chaetomium globosum* has been reported to produce structurally unique and biologically diverse secondary metabolites, mainly including cytochalasans and azaphilones [1], which have gained widespread attention from both natural product chemistry and biosynthetic perspectives. For example, several novel cytochalasans, as exemplified by armochaeglobines A and B [2], armochaetoglobins A–E [3], armochaetoglasin A [4], aureochaeglobosins A–C [5], etc., were discovered from *Chaetomium globosum* species. Most importantly, Tan and coworkers have found that the addition of 1-methyl-L-tryptophan (1-MT) into cultures of *Chaetomium globosum* 1C51 could activate the Pictet–Spenglerase (FPS) gene to enable the Pictet-Spengler reaction between 1-MT and flavipin to produce many novel alkaloids [6]. Inspired by this methodology, we have previously fed 1-methyl-L-tryptophan (1-MT) into the rice cultures of an arthropod-derived fungus *Chaetomium globosum* TW1-1 to excavate its chemical diversity, and found three novel 1-MT-derived cytochalasans, namely armochaetoglosins A–C [7]. Despite remarkable findings on the cytochalasans are frequently reported, to our surprise, the chemical space of azaphilones representing a rare family of fungal pigments characterized by a highly oxygenated pyrano-quinone bicyclic core, remains insufficiently tapped, which thus catches our attention.

Following a preliminary phytochemical investigation on structurally unique and bioactive metabolites from the EtOAc extracts of 1-MT-additive solid cultures of the title fungus, two new chlorinated azaphilones (**1** and **2**) were isolated and identified. Remarkably, chaephilone C, which was firstly isolated from a deep sea-derived fungus *Chaetomium* sp. NA-S01-R1 and identified to possess an unprecedented 6-6-5-6-fused ring system with a chlorine substitution [8], was structurally revised to **1**. Herein we describe the isolation, structure elucidation, and bioactivity evaluation of these new compounds (Fig. 1).

The 300 Erlenmeyer flasks (1 L) containing sterile solid medium (compositions: 200 g of rice, 0.42 g of 1-MT, and 200 mL of distilled water) were inoculated with the strain *Chaetomium globosum* TW1-1 and incubated at 28 °C for 21 days. The fungal mycelia as well as the rice media were exhaustively extracted with EtOH and concentrated in *vacuo* to afford a dry extract, which was suspended in water and partitioned with EtOAc to give a total extract (313 g). The EtOAc-soluble extract was then subjected to

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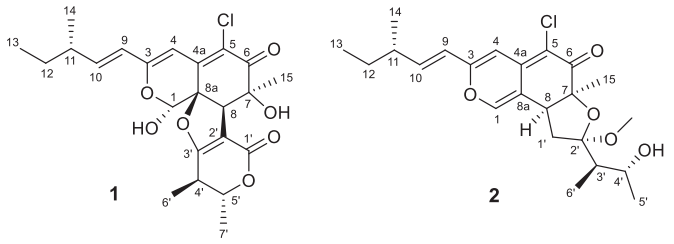

Fig. 1. Structures of compounds **1** and **2**.

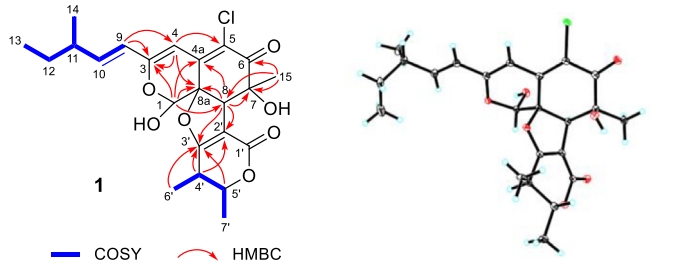

Fig. 2. Selected 2D NMR correlations of and X-ray crystal structure of compound **1**.

chromatography on a silica gel column eluted with CH_2Cl_2–MeOH (30:1–2:1) progressively to obtain five main fractions (Fr. 1–Fr. 5). Fr. 3 and Fr. 4 were separated with ODS (stepwise gradient elution from 20% to 80%, MeOH in H_2O) to yield the main subfractions 3a–3d and 4a–4d, respectively. Subfraction 3c was purified by Sephadex LH-20 (MeOH) and semi-preparative HPLC to yield compound **1** (25.3 mg, t_R 41.2 min, 62% MeCN in H_2O). Compound **2** (26.2 mg) was isolated from subfraction 4c by using Sephadex LH-20 (MeOH) and semi-preparative RP-C_{18} HPLC (t_R 47.1 min, 77% MeOH in H_2O).

Compound **1** [9] was isolated as yellow crystals. The molecular formula $C_{23}H_{27}ClO_7$ was deduced from its HRESIMS data at *m/z* 473.1333 $[M + Na]^+$ (calcd for 473.1338), which corresponded to ten degrees of unsaturation. Apart from six degrees of unsaturation occupied by eight olefinic carbons [δ_C 100.0 (CH), 102.8 (C), 124.5 (C), 124.5 (CH), 139.2 (C), 146.0 (CH), 157.4 (C), 176.8 (C)] and two carbonyl (δ_C 167.7 and 193.7) groups, the remaining four degrees of unsaturation required **1** to have a tetracyclic ring system.

The planar structure of **1** was determined based on the 2D NMR data, mainly including the ^1H–^1H COSY and HMBC spectra (Fig. 2). When further analyzing the 1D NMR data (Table 1), we found that the ^{13}C NMR data (recorded in DMSO d_6) of **1** were identical to those of chaephilone C [8], which was firstly isolated from a deep sea-derived fungus *Chaetomium* sp. NA-S01-R1 and identified to possess an unprecedented 6-6-5-6-fused ring system with a chlorine substitution. Together with their identical negative specific rotations, the above evidence suggested that both compounds shared the same planar structure and absolute configuration. Despite the relative configuration of chaephilone C had ever been defined by the ROESY data, some correlations were indeed unable to be clearly observed in the ROESY experiment [8]. More critically, the complexity of **1** containing multiple contiguous quaternary carbons and as many as seven chiral centers made it difficult to determine the absolute configuration. The above-mentioned evidence inspired us to suspect the correctness of chaephilone C.

To determine the absolute configuration of **1** to verify our speculation, the reliable crystallography experiment came into our sight. Fortunately, the recrystallization of **1** from MeOH with two drops of water furnished a high-quality crystal, which was successfully subjected to the single-crystal X-ray diffraction analysis (Fig. 2). A Flack parameter of 0.084(7) not only confirmed the planar structure of **1**, but also suggested the absolute configuration of chaephilone C to be reassigned as **1** (1*R*,7*S*,8*R*,8a*R*,9*E*,11*S*,4′*R*,5′*R*) (Fig. 3).

Compound **2** [10] was also isolated as yellow crystals. Its molecular formula, $C_{23}H_{31}ClO_5$, which was indicative of eight degrees of unsaturation, was established by the HRESIMS data at *m/z* 445.1753 ($[M + Na]^+$, calcd for 445.1752). The NMR data (Table 2)

Table 1
^1H NMR and ^{13}C NMR data (δ in ppm, *J* in Hz) for **1** and chaephilone C.

no.	**1**			**chaephilone C**	
	δ_H[a]	δ_C[a]	δ_C[b]	δ_H[c]	δ_C[c]
1	5.46 s	94.4 CH	93.0 CH	5.38 d (5.19)	93.0 CH
3		157.4 C	155.6 C		155.6 C
4	6.03 s	100.0 CH	99.5 CH	6.01 s	99.5 CH
4a		139.2 C	137.7 C		137.7 C
5		124.5 C	124.2 C		124.2 C
6		193.7 C	190.0 C		190.0 C
7		76.1 C	72.9 C		72.9 C
8	3.66 s	50.6 CH	48.6 CH	3.29 m	48.6 CH
8a		87.9 C	85.7 C		85.7 C
9	6.10 d (15.6)	124.5 CH	123.7 CH	6.18 d (15.95)	123.7 CH
10	6.43 dd (15.5, 8.1)	146.0 CH	144.2 CH	6.33 m	144.2 CH
11	2.24 m	40.0 CH	38.1 CH	2.23 m	38.2 CH
12	1.42 m	30.4 CH_2	29.1 CH_2	1.36 m	29.1 CH_2
13	0.91 t (7.5)	12.1 CH_3	12.0 CH_3	0.86 t (7.44)	12.1 CH_3
14	1.06 d (6.5)	19.9 CH_3	19.5 CH_3	1.02 d (6.74)	19.5 CH_3
15	1.46 s	27.3 CH_3	24.0 CH_3	1.31 s	24.0 CH_3
1′		167.7 C	164.5 C		164.5 C
2′		102.8 C	100.6 C		100.6 C
3′		176.8 C	175.0 C		175.1 C
4′	2.68 m	36.8 CH	35.2 CH	2.78 m	35.2 CH
5′	4.25 m	80.4 CH	78.6 CH	4.29 m	78.6 CH
6′	1.08 d (6.5)	12.1 CH_3	12.3 CH_3	0.98 d (7.19)	12.3 CH_3
7′	1.43 d (6.6)	18.5 CH_3	18.5 CH_3	1.36 d (6.39)	18.5 CH_3
OH-1				8.19 d (5.19)	
OH-7				5.72 s	

[a] Recorded in methanol d_4 at 400 MHz for ^1H- and 100 MHz for ^{13}C NMR; [b]Recorded in DMSO d_6 at 100 MHz for ^{13}C NMR; [c]Recorded in DMSO d_6 at 600 MHz for ^1H- and 150 MHz for ^{13}C NMR.

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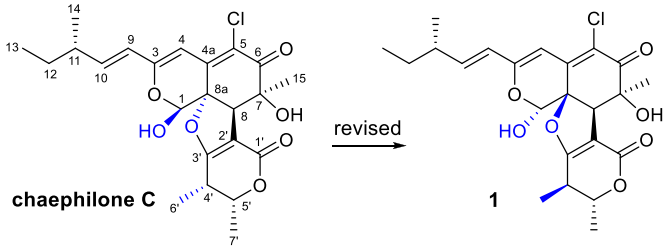

Fig. 3. Structure revision of chaephilone C to **1**.

Table 2
^{1}H NMR and ^{13}C NMR data (δ in ppm, J in Hz) for **2**.

no.	**2**	
	δ_H[a]	δ_C[a]
1	7.19 s	144.3 CH
3		157.3 C
4	6.44 s	104.7 CH
4a		140.4 C
5		109.9 C
6		189.1 C
7		85.7 C
8	3.42 dd (12.7, 6.6)	42.1 CH
8a		116.4 C
9	6.01 d (15.7)	120.5 CH
10	6.45 dd (15.7, 8.0)	146.0 CH
11	2.22 m	38.9 CH
12	1.39 m	29.3 CH_2
13	0.86 t (7.4)	11.8 CH_3
14	1.04 d (6.7)	19.5 CH_3
15	1.40 s	25.3 CH_3
1′	2.03 t (12.3); 1.90 dd (12.3, 6.6)	40.0 CH_2
2′		111.0 C
3′	2.10 m	42.1 CH
4′	3.32 m	66.9 CH
5′	1.08 d (6.2)	21.0 CH_3
6′	0.74 d (7.3)	13.1 CH_3
–OCH_3	3.26 s	48.0 CH_3

[a] Measured in $CDCl_3$ at 400 MHz for ^{1}H- and 100 MHz for ^{13}C NMR.

of **2** highly resembled those of chaetomugilin L [11]. Detailed interpretation of the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 4) of **2** revealed the same core structure for both substances, with the difference being that a C-3′–C-4′ double bond in chaetomugilin L was replaced by an sp^3 methine (δ_C 42.1; C-3′) and an oxygenated methine (δ_C 66.9; C-4′) in **2**, which was supported by the ^{1}H–^{1}H COSY cross-peaks of H_3-6′/H-3′/H-4′/H_3-5′. An obvious NOESY correlation of H-8/H_3-15 revealed that H-8 and H_3-15 should be on the same side with α-orientations. With regard to the relative configurations of C-2′, C-3′, C-4′, and C-11, they could not be defined by analyzing the NORSY data alone. To our excitement, **2** could also provide a suitable crystal in MeOH mixed with two drops of water, which enabled us to determine its absolute configuration as 7*S*,8*S*,9*E*,11*S*,2′*R*,3′*R*,4′*R* through the single-crystal X-ray diffraction analysis (Fig. 4).

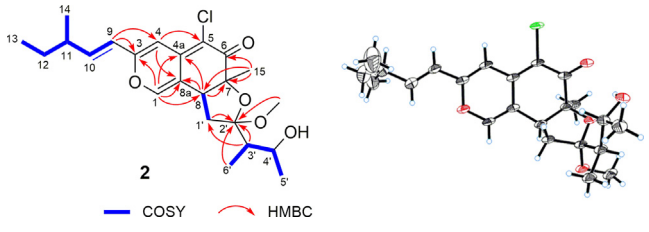

Fig. 4. Selected 2D NMR correlations of and X-ray crystal structure of compound **2**.

Compounds **1** and **2** were evaluated for the anti-inflammatory activity against the nitric oxide (NO) production in LPS-induced RAW264.7 macrophages [12], and found to show moderate anti-inflammatory activity with IC_{50} values of 15.12 ± 1.48 and 20.65 ± 0.95 μM, respectively. In addition, the α-glucosidase inhibitory activity [13] for compounds **1** and **2** were also screened, however, none of them was active at a concentration of 40 μM.

In conclusion, two new chlorinated azaphilones (**1** and **2**) with moderate anti-inflammatory activity were obtained from the 1-MT-additive solid cultures of an arthropod-derived fungus *Chaetomium globosum* TW1-1. The multiple contiguous quaternary carbons and chiral centers made the structure elucidation for absolute structures of **1** and **2** rather challenging, in which the nuclear magnetic resonance data analyses were helpless, and fortunately, the enabled crystallography experiment solved this problem and also helped us to revise the absolute structure of chaephilone C featuring an unprecedented 6-6-5-6-fused ring system with a chlorine substitution to be that of **1**.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151516.

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[9] Compound **1**: yellow crystals; $[\alpha]_D^{20}$: −317 (c = 0.1, MeOH); UV (MeOH) λ_{max} (log ε) = 202 (4.09), 247 (4.10) and 365 (4.32) nm; IR ν_{max} = 3427, 2967, 1686, 1643, 1577, 1397, 1174, 1022, 922 and 731 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) = 232 (−18.3), 252 (+5.3) and 343 (−8.8) nm; For ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1; HRESIMS [M + Na]$^+$ m/z 473.1333 (calcd for $C_{23}H_{27}ClO_7Na^+$, 473.1338).
[10] Compound **2**: yellow crystals; $[\alpha]_D^{20}$: +27 (c = 0.1, MeOH); UV (MeOH) λ_{max} (log ε) = 203 (4.04), 225 (4.07), 294 (3.90) and 389 (4.15) nm; IR ν_{max} = 3436, 2970, 1618, 1563, 1523, 1383, 1258, 1022 and 877 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) = 204 (+7.0), 220 (−5.2), 246 (+2.8), 334 (−7.7) and 388 (+4.7) nm; For ^{1}H NMR

(400 MHz) and ^{13}C NMR (100 MHz) data, see Table 2; HRESIMS $[M + Na]^+$ m/z 445.1753 (calcd for $C_{23}H_{31}ClO_5Na^+$, 445.1752).

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