SUPPLEMENTARY MATERIAL

Potential allelopathic azaphilones produced by the endophytic *Chaetomium globosum* TY1 inhabited in *Ginkgo biloba* using the one strain–many compounds method

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On the basis of the OSMAC strategy, 7 azaphilones, including Chaetomugilin A (**1**), D (**2**), S (**3**), I (**4**), J(**5**), Q (**6**), and O (**7**), were isolated from the endophytic *Chaetomium globosum* TY1. Their structures were identified by NMR and HRESIMS spectrometry data. All azaphilones were evaluated for plant growth regulation using eight species of herbaceous plant seeds seedling growth bioassay, which showed the plant growth influence of the seedling. Among these compounds tested, Chaetomugilin O (**7**) with tetrahydrofuran, exhibited higher response index and lower IC₅₀ values than positive control glyphosate, a broad-spectrum systemic herbicide. **1**, **2** and **3** also showed better or similar inhibit activity to glyphosate. The structure–allelopathic activity relationship analysis of these isolated azaphilones indicates that both tetrahydrofuran and tetrahydrofuran combine with lactones ring groups give potent inhibition of seedling growth. Chaetomugilin O and Chaetomugilin A, D, S could be used to develop a natural eco-friendly herbicide.

Nomenclature: *Chaetomium globosum* TY1.

Keywords: herbicides, allelochemicals, azaphilone, phytotoxicity.

Experimental

Fungi and fermentation. The fungal strain *Chaetomium globosum* TY1 was separated from the fresh barks of the host *Ginkgo biloba*, a medicinal plant growing in Linyi, Shandong province, China. It was authenticated by Prof. H.Y. Pan based on morphological studies and has been deposited at College of Plant Science, Jilin University. After growing on Potato Dextrose Ager medium at 25 °C for 4 days, the fresh mycelium of this fungal strain was inoculated in Potato Dextrose Broth, the pH was adjusted to 6.0 before autoclaving. Fermentation was carried out in 30 × 2800 mL flasks each containing 1500 mL medium on a rotary shaker at 150 rpm/min, 25 °C for 23 days. This fungus was also grown on a sterilized moistened rice medium in Roux asks (100 g/ask × 300) at 25 °C for 53 days to give green moldy rice.

Extraction and Isolation. The fungal cultures grown on rice medium were ultrasonically extracted four times with acetone. The solvent was removed under reduced pressure to give a crude extract (125g). The extract was subjected to column chromatography on silica gel with CH₂Cl₂/CH₃OH (100:1, 50:1, 20:1 and 10:1) to give four fractions A–D. Fraction C (17.3 g) was chromatographed over Sephadex LH-20 (CH₃OH), one subfraction C-4-4 was repeatedly chromatographed over silica column (CH₂Cl₂/CH₃OH) and reversed-phase (ODS) column to afford the compound **1** (56.8 mg) and **2** (68.0 mg), and another subfraction C-2-2 was repeatedly subjected over silica column (CH₂Cl₂/CH₃OH) to afford **3** (48.7 mg). Fraction D (29.2 g) was further separated by Sephadex LH-20 (CH₂Cl₂/CH₃OH = 4:6) and one subfraction D-4-2 was repeatedly chromatographed over silica column (CH₂Cl₂/CH₃OH) and reversed-phase (ODS) column to harvest compound **4** (56.2 mg) and **5** (46.0 mg).

The PDB culture broth was extracted with EtOAc three times to yield 68 g of extract. The

crude extract was then subjected to a silica gel column and eluted with a CH₂Cl₂/CH₃OH mixture (100:1, 50:1, 20:1, and 10:1) to give three fractions (E-H). Fraction G (6.8g) was fractionated on silica gel (CH₂Cl₂/CH₃OH), and the subfractionG-2 was purified by reversed-phase (ODS) column to produce compound **7** (53.1 mg), subfractionG-3 was repeatedly chromatographed over silica column (CH₂Cl₂/CH₃OH) and reversed-phase (ODS) column to afford the compound **6**(60.2 mg).

Identification of fungal metabolitesChemical investigations of *C. globosum* TY1 crude extracts obtained from two different media led to the isolation of a series of azaphilones (Figure 1) by multiple chromatographic procedures. ¹H NMR and ¹³C NMR data of compounds in CDCl₃ see Table S1 and S2. Chaetomugilin A (**1**): Yellow powder; HRESIMS m/z 451.1516 [M+H]⁺ (calcd for C₂₃H₂₈³⁵ClO₇, 451.1518); Chaetomugilin D (**2**): Yellow gum; HRESIMS m/z435.1551 [M+H]⁺ (calcd for C₂₃H₂₈³⁵ClO₆, 435.1569); Chaetomugilin S (**3**): Yellow powder; HRESIMS m/z 435.1577 [M+H]⁺ (calad for C₂₃H₂₈³⁵ClO₆, 435.1569); Chaetomugilin I (**4**): Yellow powder; HRESIMS m/z 407.1607 [M+H]⁺ (calcd for C₂₂H₂₈³⁵ClO₅, 407.1620); Chaetomugilin J (**5**) Yellow powder; HRESIMS m/z 391.1662 [M+H]⁺ (calcd for C₂₂H₂₈³⁵ClO₄, 391.1671); Chaetomugilin Q (**6**) Yellow powder; HRESIMS m/z 423.1571 [M-H]⁻ (calcd for C₂₂H₂₈³⁵ClO₆, 423.1580); Chaetomugilin O (**7**) Yellow powder; HRESIMS m/z 417.1459 [M+H]⁺ (calcd for C₂₃H₂₆³⁵ClO₅, 417.1463); Their structures were identified on the basis of comparison of their ¹H NMR, ¹³C NMR and HR-MS data with those reported (Yamada et al. 2008; Qin et al. 2009; Yamada et al. 2012; Muroga et al. 2009; Yamada et al. 2011; Takahashi et al. 1990).

Allelopathic Bioassay.The seeds of eight herbaceous plants, *Cucumissativus* (cucumber), *Brassica campestris* (rape), *Eruca sativa* (rucola),*Lactuca sativa* (lettuce), *Daucuscarota* (carrot), *Scrophularianingpoensis* (summer radish), *Spinaciaoleracea* (spinach), *Brassica rapa* (bokchoy), were used for the bioassay. The procedure was conducted according to the reported protocol (Zhang et al.2013). The plant seeds was washed with running water for 2 h, soaked in 0.5% KMnO₄ for 15 min, and washed until they were colorless. The compounds, positive control and blank solvent acetone, were added to 12-well plates with filter paper to final concentrations of 10, 20, 50, 100 and 200 ppm. After the evaporation of acetone, the plant seeds were sown in the microdishes of 12-well plates and irrigated with deionized water. Triplicate experiments were conducted. The plates were then incubated at 24 °C for 96 h, and the germination rates (GR) were calculated according to eq 1. Germinate inhibition rate (GIR) were calculated according to eq 2. Allelopathic effects [response index (RI)] were calculated according to eq 3.

germination rate (%) =(number of germinated seeds)/(total number of seeds) (1)

germinate inhibition rate (%)=(germination rate of CK - germination rate of control)/germination rate of CK (2)

If T > C , then RI = 1 – C / T ; if T < C , then RI = T / C–1 (3)

where T is the length of the treatment, C is the length of the blank control, and RI is the response index. Data were evaluated via Probit SPSS Analysis (SPSS 13.0 for Windows, SPSS Inc., 2004) for determination of IC₅₀ and 95 % confidence intervals.

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Table S1. ¹H NMR data of compounds (300 MHz, CDCl₃)

Position	δ_{H} (J/Hz)						
	1	2	3	4	5	6	7
1	7.27 s	7.27 s	7.71 s	7.48 s	7.47 s	7.42 s	7.33 s
4	6.57 s	6.55 s	6.53 s	6.47 s	6.45 s	6.47 s	6.49 s
8	2.98 d (10.0	2.97 d 10.1	3.30 d 9.0	3.48 dd 10.5, 3.0	3.48 dd 10.2, 2.2	3.46 dd 9.5, 3.5	4.11 d 12.0
9	6.15 d 15.0	6.04 d 15.6	6.06 d 15.5	6.12 d 16.0	6.02 d 15.5	6.11 d 15.5	6.04 d 15.7
10	6.61 dd (15.0, 6.2	6.52 dd 15.6, 6.5	6.52 dd 15.5, 6.5	6.58 dd 16.0, 7.2	6.48 dd 15.5, 6.5	6.59 dd 15.5, 8.0	6.50dd 15.7, 7.0
11	2.45 sex 6.2	2.23 m 6.8	2.26 m 6.5	2.44 sex 7.2	2.24 m 6.5	2.44 m 6.5	2.25 sept 7.0
12	3.81 br s	1.42 m	1.43 m	3.80 q 7.2	1.42 qd 7.5, 6.5	3.81 qd 6.5, 5.8	1.41 m
13	1.20 d 6.2	0.87 t 7.3	0.90 t 7.5	1.19 d 6.5	0.89 t 7.5	1.19 d 6.5	0.89 t 7.0
7-Me	1.40 s	1.39 s	1.38 s	1.33 s	1.33 s	1.31 s	1.63 s
11-Me	1.13 d 6.2	1.10 d 6.4	1.08 d 6.5	1.12 d 7.2	1.07 d 6.5	1.12 d 6.5	1.07 d 7.0
1' A				2.62 dd 17.5, 10.5	2.62 dd 17.3, 10.2	2.39 dd 18.0, 9.5	
1' B				3.27 dd 17.5, 3.0	3.28 dd 17.3, 2.2	3.2 dd 18.0, 3.5	
2'	3.06 d 10.0	3.06 d 10.1	3.04 d 9.0				4.37 d 12.0
3'						2.41 dq 7.5, 7.0	
4'	1.90 dq 10.3, 6.2	1.87 dq 10.1, 6.9	1.79 dq 10.0, 7.0	6.66 q 7.5	6.66 q 7.1	3.86 dq 7.5, 6.1	
5'	4.30 dq	4.28 dq	4.34 dq	1.81 d	1.80 d	1.13 d	6.80q

	10.3, 6.5	10.1, 6.4	10.0, 6.2	7.5	7.1	6.1	
6'	1.41 d	1.40 d	1.35 d				1.94 d
	6.5	6.4	6.2				7.0
4'-Me	1.13 d	1.11 d	1.11 d				1.84s
	6.2	6.2	7.0				
3'-Me				1.73 s	1.73 s	1.04 d	
						7.0	

Table S2. ¹³C NMR data of compounds(125 MHz, CDCl₃)

Position	δ_c (J/Hz)						
	1	2	3	4	5	6	7
1	145.67	145.6	147.0	145.7	145.7	145.3	145.8
3	157.11	157.7	157.4	156.6	157.2	156.8	157.7
4	105.47	105.0	104.8	105.0	104.5	105.0	104.6
4a	140.07	140.4	140.6	141.6	141.8	141.5	140.5
5	110.43	110.1	109.9	106.8	106.5	107.0	109.6
6	189.25	189.2	189.1	191.9	191.8	191.7	184.7
7	83.98	84.0	84.1	74.1	74.1	74.1	83.5
8	50.55	50.6	47.7	40.6	40.5	40.6	43.4
8a	114.29	114.3	115.2	119.6	119.5	119.6	113.7
9	122.10	120.2	120.3	122.4	120.4	122.2	120.1
10	142.52	146.9	146.3	141.8	146.1	142.0	147.0
11	44.32	38.9	38.8	44.2	38.8	44.2	38.9
12	70.90	29.2	29.2	70.9	29.2	70.9	29.1
13	20.52	11.7	11.7	20.4	11.7	20.4	11.7
7-Me	23.23	23.2	23.8	26.8	26.8	26.6	23.2
11-Me	14.85	19.4	19.4	14.8	19.4	14.7	19.3
1'	170.50	170.6	171.9	34.8	34.9	40.9	168.3
2'	58.24	58.3	57.5	199.4	199.4	213.7	51.6
3'	104.17	104.2	103.5	138.0	138.0	54.0	191.4
4'	44.89	44.9	46.1	138.0	138.0	69.8	137.5
5'	76.89	76.9	77.1	14.7	14.7	20.9	144.7
6'	18.70	18.7	18.0				15.4
4'-Me	8.79	8.8	8.7	11.0	11.0	13.6	11.3

Table S3. IC₅₀ of isolated compounds and glyphosate against herbaceous plant seeds.

Comp	IC ₅₀ ppm
ound	95% CL ^a

	<i>C. sativus</i>	<i>B. campestris</i>	<i>E. sativa</i>	<i>L. sativa</i>	<i>D. carota</i>	<i>S. ningpoe nsis</i>	<i>S. oleracea</i>	<i>B. rapa</i>
1	41.94 37.47- 46.95	70.14 65.43- 75.19	76.84 67.01- 88.12	11.30 9.711- 13.15	3.056 1.340- 6.966	92.02 87.13- 97.18	47.63 43.73- 51.87	9.956 8.175- 12.12
2	46.29 41.43- 51.71	64.03 60.16- 68.14	82.01 75.10- 89.56	10.73 10.49- 10.97	< 10 ^b	74.33 68.45- 80.71	19.21 17.67- 20.88	16.54 15.16- 18.03
3	46.33 40.65- 52.80	66.00 62.23- 70.01	71.08 65.22- 77.46	9.597 9.192- 10.02	< 10	77.83 72.00- 84.12	19.10 17.60- 20.73	14.99 13.41- 16.75
4	292.5 197.5- 433.4	122.0 105.7- 140.8	95.66 89.70- 102.0	331.6 238.4- 461.2	202.9 170.0- 242.2	75.17 69.19- 81.68	51.00 46.11- 56.41	95.89 85.04- 108.1
5	71.93 62.11- 83.29	71.18 63.56- 79.70	84.06 77.40- 91.28	139.8 121.2- 161.2	64.61 58.47- 71.40	98.51 95.25- 101.9	56.68 51.16- 62.81	90.94 78.63- 105.2
6	230.3 203.5- 260.7	433.1 314.7- 596.2	509.7 271.7- 956.1	131.9 121.1- 143.8	63.97 58.05- 70.50	189.3 179.2- 199.9	71.10 61.42- 82.31	216.3 188.4- 248.4
7	33.08 28.74- 38.07	57.46 53.69- 61.51	51.33 49.52- 53.20	< 10	< 10	73.85 66.06- 82.57	13.07 11.45- 14.91	11.18 10.35- 12.07
GP ^c	43.46 37.25- 50.71	81.52 72.22- 92.01	62.56 56.02- 69.85	71.00 61.08- 82.54	73.42 63.81- 84.48	39.56 33.44- 46.80	36.31 32.74- 40.27	19.03 15.52- 23.33

a. P<0.05, level of significance.

b. Germination inhabited at all tested concentrations (10-200 ppm).

c. Glyphosate (positive control).

Figure S2. germinate inhibition activity of isolated compounds and glyphosate against herbaceous plant seeds.

Figure S3. Inhibition by isolated compounds and glyphosate of the elongation of herbaceous plant shoot.

Figure S4. Inhibition by isolated compounds and glyphosate of the elongation of herbaceous plant root.

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