SUPPLEMENTARY MATERIAL

Anti-phytopathogen, multi-target acetylcholinesterase inhibitory, and antioxidant activities of metabolites from endophytic *Chaetomium globosum*

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Fourteen metabolites with various structure types were isolated from endophytic *Chaetomium globosum*. Five compounds were separated from genus *Chaetomium* for the first time. Some compounds exhibited remarkable inhibition against phytopathogenic fungi causing root rot of *Panax notoginseng*. Compounds **1-5** had significant DPPH free radical-scavenging activity. Compounds **3** and **5** indicated significant inhibitions against the acetylcholinesterase (AChE). From preliminary structure-activity relationship (SAR), it was found that the oxygenic five membered ring of **3** and **5** was crucial in the anti-AChE activity. These structures provide new templates for the potential treatment and management of plant diseases and Alzheimer disease.

Keywords: *Chaetomium globosum*, metabolite, anti-phytopathogen, anti-AChE, antioxidant

Experimental

General experimental procedures

Silica gel (200–300 mesh; Qingdao Marine Chemical Group Co., Qingdao, China), and Sephadex LH-20 (GE Healthcare Co., Buckinghamshire, UK) were used for column chromatography. The semipreparative HPLC (Agilent series 1200) was performed on YMC C18 column (250 mm × 10 mm, 5 μm). 1D and 2D NMR spectra were obtained on a Bruker AVANCE 400MHz NMR instrument (Bruker, Karlsruhe, Germany). MS spectra were recorded with Agilent G3250AA (Agilent, Santa Clara, USA).

Fungal material

The endophytic fungus PH30461 was isolated by using PDA medium from the seeds of *P. notoginseng* that was collected at the Wenshan, Yunnan, China, and was identified as *C. globosum* by ITS gene sequence. The sequence data derived from the fungal strain have been submitted to and deposited at GenBank under accession no.KP714386. A BLAST search result showed that the sequence was the most similar (100%) to the sequence of *C. globosum* (compared to JX981455). The strain is preserved at Yunnan Institute of Microbiology, Yunnan University, Kunming, China.

Fermentation, extraction, and isolation

Fungus PH30461 was cultured in 500mL Erlenmeyer flasks each containing 200mL potato dextrose broth (PDB) seed culture (1000 mL water, 20 g glucose, 200 g potato). After 3 days of incubation at 28 °C on a rotary shaker (125 r.p.m.), 10mL portion of the culture was inoculated into 1000 mL Erlenmeyer flasks each contained of 300 mL PDB fermentation medium for 7 days at 28 °C on a rotary shaker (125 r.p.m.). The fermented whole broth 50 L was filtered through hospital gauze to separate the culture broth and mycelia. The former was extracted three times with EtOAc to give a crude residue (46 g). The extract was subjected to column chromatography over silica gel eluted with stepwise $\text{CHCl}_3/\text{MeOH}$ gradient (CHCl_3 , $\text{CHCl}_3/\text{MeOH}$ 50:1, v/v; $\text{CHCl}_3/\text{MeOH}$ 30:1, v/v, $\text{CHCl}_3/\text{MeOH}$ 10:1, v/v; MeOH) to yield 7 fractions (Fr1-7) on the basis of TLC analysis. Then Fr 2 and Fr 4 were separated on Sephadex LH-20 (MeOH) to give 4 fractions (Fr 2-1 to 2-4 and Fr 4-1 to 4-4) respectively. In the further purification, silicon gel column chromatography with petroleum ether/ethyl acetate, $\text{CHCl}_3/\text{MeOH}$; reversed-phase (RP) silica gel C-18 with $\text{H}_2\text{O}/\text{MeOH}$; RPHPLC with $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ were used. Finally, chaetomugilins A (**6**, 45 mg) and D (**7**, 40 mg) were obtained from Fr 2-1, chaetoglobosins A (**8**, 30 mg), B (**9**, 15 mg), chaetoglobosin E (**10**, 3.5 mg), chaetoglobosins F (**11**, 25 mg) and F_{ex} (**12**, 1 mg), penochalasins F (**13**, 4 mg) and G (**14**, 1 mg), were obtained from Fr 2-2; flavipin (**1**, 40 mg) was obtained from Fr 4-4. Fr 6 was separated into two parts as Fr 6S and Fr 6L by filtration. From the Fr 6S, epicoccolide B (**5**, 20 mg) was obtained after a

reversed-phase silicon gel C-18 column chromatography. Fr 6L was eluted with petroleum ether/ethyl acetate by silica gel column chromatography and was further purified by reversed-phase silica gel C-18 column, and Sephadex LH-20 (MeOH) chromatography to afford epicoccone (**2**, 10 mg), 3-methoxyepicoccone (**3**, 26 mg) and epicoccolide A (**4**, 9 mg).

Spectra data

Flavipin (**1**): Yellow solid. ESI-MS m/z 195 $[M-H]^-$. 1H NMR (acetone- d_6 , 400MHz) δ : 10.58 (1H, s, H-8), 10.39 (1H, s, H-9), 2.55 (1H, s, H-7). ^{13}C NMR (acetone- d_6 , 100MHz) δ : 196.6 (C-8), 191.9 (C-9), 150.7 (C-5), 149.4 (C-3), 135.6 (C-4), 128.1 (C-1), 123.7 (C-6), 111.2 (C-2), 9.3 (C-7).

Epicoccone (**2**): Maple solid. 1H NMR (DMSO- d_6 , 400MHz) δ : 5.09 (2H, s, H-3), 2.33 (3H, s, H-8). ^{13}C NMR (DMSO- d_6 , 100MHz) δ : 171.9 (C-1), 145.3 (C-6), 140.3 (C-4), 137.3 (C-5), 126.6 (C-3a), 116.4 (C-7), 112.8 (C-7a), 66.8 (C-3), 10.0 (C-8).

3-methoxyepicoccone (**3**): 1H NMR (MeOD, 400MHz) δ : 6.26 (1H, s, H-3), 3.48 (3H, s, 3-OCH₃), 2.12 (3H, s, H-8). ^{13}C NMR (MeOD, 100MHz) δ : 169.8 (C-1), 151.6

(C-6), 142.6 (C-4), 134.8 (C-5), 134.2 (C-3a), 112.9 (C-7), 103.5 (C-7a), 102.6 (C-3), 54.3 (3-OCH₃), 10.0 (C-8).

Epicoccolides A (**4**): Yellow solid. ¹H NMR (DMSO-d₆, 400MHz) δ : 10.31 (1H, s, H-18), 6.81 (1H, s, H-10), 6.36 (1H, s, H-2), 2.31 (3H, s, H-17), 2.25 (3H, s, H-19). ¹³C NMR (DMSO-d₆, 100MHz) δ : 197.0 (C-9), 191.2 (C-18), 152.9 (C-5), 148.6 (C-7), 144.2 (C-14), 138.4 (C-15), 135.8 (C-16), 132.5 (C-6), 126.6 (C-3), 121.7(C-12), 121.7 (C-13), 115.4 (C-4), 112.9 (C-11), 104.1 (C-8), 90.0 (C-2), 68.7 (C-10), 11.8 (C-17), 10.2 (C-19).

Epicoccolides B (**5**): Yellow solid. ESI-MS m/z 357 [M-H]⁻. ¹H NMR (DMSO-d₆, 400MHz) δ : 10.37 (1H, s, H-8), 9.45 (1H, s, H-15), 7.45 (1H, s, H-3), 2.57 (3H, s, H-17), 2.00 (3H, s, H-16). ¹³C NMR (DMSO-d₆, 100MHz) δ : 194.6 (C-15), 189.9 (C-8), 152.0 (C-2), 151.6 (C-13), 150.2 (C-14), 142.6 (C-7a), 141.2(C-6), 137.8 (C-7), 132.8 (C-12), 127.1(C-5), 125.0 (C-9), 123.1 (C-3a), 118.8 (C-11), 116.5 (C-4), 112.5 (C-10), 109.0 (C-3), 12.8 (C-16), 11.1 (C-17).

Chaetomugilins A (**6**): Yellow solid. ^1H NMR (CDCl_3 , 400MHz) δ : 7.27 (1H, s, H-1), 6.61 (1H, m, H-10), 6.55 (1H, s, H-4), 6.14 (1H, d, $J = 16.0\text{Hz}$, H-9), 4.29 (1H, m, H-5'), 3.80 (1H, m, H-12), 3.00 (1H, H-8, H-2'), 2.45 (1H, m, H-11), 1.86 (1H, m, H-4'), 1.36 (6H, 7- CH_3 , H-6') , 1.17 (3H, d, $J = 6.4\text{Hz}$, H-13), 1.10 (6H, 4'- CH_3 , 11- CH_3). ^{13}C NMR (CDCl_3 , 100MHz) δ : 189.2 (C-6), 170.8 (C-1'), 157.3 (C-3), 145.6 (C-1), 142.7 (C-10), 140.4 (C-4a), 122.1 (C-9), 114.5 (C-8a), 110.3 (C-5), 105.4 (C-3'), 104.0 (C-4), 83.7 (C-7), 76.7 (C-5'), 70.9 (C-12), 58.3 (C-2'), 50.4 (C-8), 45.0 (C-4'), 44.2 (C-11), 23.4 (7- CH_3), 20.2 (C-13), 18.7 (C-6'), 14.9 (11- CH_3), 8.8 (4'- CH_3).

Chaetomugilins D (**7**): Yellow solid. ^1H NMR (CDCl_3 , 400MHz) δ : 7.27 (1H, s, H-1), 6.52 (1H, m, H-4), 6.48 (1H, m, H-10), 6.05 (1H, d, $J = 16.0\text{Hz}$, H-9), 4.29 (1H, m, H-5'), 3.04 (1H, d, $J = 10.0\text{Hz}$, H-12), 2.98 (1H, d, $J = 10.0\text{Hz}$, H-8), 2.24 (1H, m, H-11), 1.88 (1H, m, H-4'), 1.38-1.45 (7H, 7- CH_3 , H-6', H-12) , 1.12 (3H, d, $J = 6.8\text{Hz}$, 4'- CH_3), 1.06 (3H, d, $J = 6.4\text{Hz}$, 11- CH_3), 0.88 (3H, t, $J = 7.2\text{Hz}$, H-13). ^{13}C NMR (CDCl_3 , 100MHz) δ : 189.2 (C-6), 170.8 (C-1'), 157.7 (C-3), 146.8 (C-10), 145.6 (C-1), 140.5 (C-4a), 120.2 (C-9), 114.4 (C-8a), 110.1 (C-5), 105.0 (C-4), 104.1 (C-3'), 83.8 (C-7), 76.9 (C-5'), 58.3 (C-2'), 50.5 (C-8), 45.0 (C-4'), 38.9 (C-11), 29.2 (C-12),

23.4 (7-CH₃), 19.4 (11-CH₃), 18.7 (C-6'), 11.7 (C-13), 8.7 (4'-CH₃).

Cheatoglobosin A (**8**): Yellow solid. ESI-MS m/z 529 $[M+H]^+$. ^1H NMR (MeOD, 400MHz) δ : 7.46 (1H, m, H-4'), 7.24 (1H, m, H-7'), 7.69-7.00 (2H, m, H-5',6'), 6.93 (1H, s, H-2'), 6.11 (1H, d, $J = 16.4\text{Hz}$, H-21), 6.03 (1H, dd, $J = 15.2; 9.6\text{Hz}$, H-13), 5.50 (1H, d, $J = 9.2\text{Hz}$, H-14), 4.98 (1H, m, H-17), 3.89 (1H, m, H-19), 2.85 (1H, m, H-4), 2.82 (1H, m, H-7), 2.75, 2.22 (2H, m, H-10), 2.43 (1H, br, H-16), 2.00, 1.71 (2H, m, H-15), 1.97 (1H, m, H-8), 1.67 (1H, m, H-5), 1.31 (3H, s, 18-CH₃), 1.27 (3H, s, H-12), 1.05 (3H, d, $J = 7.2\text{Hz}$, 16-CH₃), 0.97 (3H, d, $J = 6.8\text{Hz}$, H-11). ^{13}C NMR (MeOD, 100MHz) δ : 202.8 (C-20), 199.8 (C-23), 176.4 (C-1), 141.8 (C-17), 138.9 (C-1'a), 136.9 (C-21), 135.1 (C-22), 134.6 (C-8), 134.2 (C-14), 130.5 (C-13), 129.7 (C-3'a), 126.6 (C-2'), 123.3 (C-5'), 120.9 (C-6'), 120.5 (C-4'), 113.5 (C-7'), 110.4 (C-3'), 83.8 (C-19), 65.9 (C-6), 64.5 (C-7), 60.2 (C-9), 55.0 (C-3), 51.0 (C-8), 48.7 (C-4), 43.7 (C-15), 38.5 (C-22), 34.5 (C-10), 34.0 (C-21), 22.1 (16-Me), 20.6 (C-12), 14.0 (C-11), 11.6 (18-Me).

Cheatoglobosin B (**9**): Yellow solid. ESI-MS m/z 529 $[M+H]^+$. ^1H NMR (CDCl_3 , 400MHz) δ : 8.19 (1H, br, 1'-NH), 7.64 (1H, d, $J = 16.4\text{Hz}$, H-22), 7.41 (1H, d, $J = 7.6\text{Hz}$, H-4'), 7.28 (1H, d, $J = 8.4\text{Hz}$, H-7'), 7.12 (1H, dd, $J = 7.2\text{Hz}$, H-5'), 7.05 (1H, dd, $J = 7.6\text{Hz}$, H-6'), 6.90 (1H, d, $J = 2.0\text{Hz}$, H-2'), 6.66 (1H, d, $J = 16.4\text{Hz}$, H-21), 6.10 (1H, dd, $J = 15.2; 10\text{Hz}$, H-13), 5.90 (1H, 1'-NH), 3.86 (1H, 19-OH), 3.76 (1H, br, H-7), 3.48 (1H, br, m, H-3), 3.32 (1H, 7-OH), 2.82, 2.59 (2H, m, H-10), 2.43 (1H, br, H-16), 2.24, (1H, br, d, $J = 12.4\text{Hz}$, H-8), 2.03 (3H, m, H-15,H-4), 1.66 (3H, s, H-11), 1.57 (3H, s, H-12), 1.29 (3H, s, 18-CH₃), 0.97 (3H, d, $J = 7.2\text{Hz}$, 16-CH₃). ^{13}C NMR (CDCl_3 , 100MHz) δ : 201.4 (C-23), 197.5 (C-20), 173.2 (C-1), 140.2 (C-17), 137.3 (C-21), 136.5 (C-1'a), 136.1 (C-22), 133.2 (C-14), 132.4 (C-18), 127.9 (C-13), 127.0 (C-3'a), 126.3 (C-5), 123.1 (C-2'), 122.6 (C-5'), 120.0 (C-6'), 118.5 (C-4'), 111.6 (C-7'), 111.1 (C-3'), 82.1 (C-19), 68.8 (C-7), 61.5 (C-9), 58.4 (C-4), 52.7 (C-3), 47.9 (C-8), 41.6 (C-15), 33.2 (C-10), 32.4 (C-16), 21.2 (16-CH₃), 18.0 (C-12), 14.0 (C-11), 10.8 (18-CH₃).

Cheatoglobosin E (**10**): Yellow solid. ^1H NMR ($\text{DMSO}-d_6$, 500MHz) δ : 10.82 (1H,

1'-NH), 8.01(1H, 2-NH), 7.31(1H, d, $J = 7.8\text{Hz}$, H-4'), 7.25 (1H, d, $J = 8.1\text{Hz}$, H-7'), 6.99 (1H, dd, H-5'), 6.97 (1H, d, $J = 2.1\text{Hz}$, H-2'), 6.89 (1H, dd, H-6'), 6.19 (1H, d, $J = 9.5\text{Hz}$, H-17), 6.15 (1H, m, H-13), 4.97 (1H, m, H-14), 4.82 (1H, 20-OH), 4.68 (1H, m, H-20), 3.52 (1H, m, H-7), 2.81, 1.91 (2H, m, H-22), 2.70 (1H, m, H-10), 2.63 (1H, m, H-4), 2.27, 1.67 (2H, m, H-15), 2.26, 1.68 (2H, m, H-21), 1.94 (1H, m, H-8), 1.62(3H, s, 18-CH₃), 1.42 (3H, s, H-11), 1.01 (3H, s, H-12), 0.92 (3H, d, $J = 6.5\text{Hz}$, 16-CH₃). ¹³C NMR (DMSO-*d*₆, 125MHz) δ : 210.5 (C-19), 204.4 (C-23), 174.3 (C-1), 148.6 (C-17), 136.5 (C-1'a), 135.4 (C-18), 133.8 (C-14), 133.8 (C-6), 128.8 (C-13), 127.4 (C-3'a), 125.8 (C-5), 123.9 (C-2'), 121.4 (C-5'), 118.8 (C-6'), 118.4 (C-4'), 111.9 (C-7'), 110.3 (C-3'), 70.5 (C-20), 68.0(C-7), 62.0 (C-9), 57.5 (C-3), 51.3 (C-8), 49.6 (C-4), 42.2 (C-15), 36.8 (C-22), 33.3 (C-16), 32.1 (C-10), 30.8 (C-21), 20.1 (16-CH₃), 17.2 (C-12), 15.0 (C-11), 12.4 (18-CH₃).

Cheatoglobosin F(**11**): White solid. ESI-MS m/z 531 [M+H]⁺. ¹H NMR (CDCl₃, 400MHz) δ : 8.62 (1H, br, H-1'), 7.49 (1H, d, $J = 8.0\text{Hz}$, H-4'), 7.38 (1H, d, $J = 8.0\text{Hz}$, H-7'), 7.20 (1H, dd, H-5'), 7.15 (1H, dd, H-6'), 6.98 (1H, d, $J = 2.0\text{Hz}$, H-2'), 6.35 (1H, m, H-13), 6.12 (1H, d, $J = 8.4\text{Hz}$, H-17), 5.25 (1H, m, H-14), 4.68 (1H, m, H-20), 3.91 (1H, d, $J = 6.4\text{Hz}$, 20-OH), 3.49 (1H, H-3), 2.86, 2.66(2H, m, H-22),

2.85 (1H, H-16), 2.85, 2.66 (2H, H-10), 2.79 (1H, H-7), 2.65 (1H, H-4), 2.38, 2.08 (2H, H-15), 2.23 (1H, m, H-8), 1.82 (4H, H-5, 18-CH₃), 1.70, 1.65 (2H, m, H-21), 1.22 (3H, s, H-12), 1.14 (3H, d, $J = 7.2\text{Hz}$, H-11), 1.05 (3H, d, $J = 7.2\text{Hz}$, 16-CH₃).

¹³C NMR (CDCl₃, 100MHz) δ : 208.4 (C-19), 203.7 (C-23), 175.0 (C-1), 149.6 (C-17), 136.5 (C-1'a), 134.4 (C-18), 133.6 (C-14), 128.8 (C-13), 127.2 (3'a), 123.5 (C-2'), 122.6 (C-5'), 120.1 (C-6'), 118.3 (C-4'), 111.8 (C-7'), 110.4 (C-3'), 72.0 (C-20), 64.7 (C-9), 61.9 (C-7), 57.5 (C-6), 52.7 (C-3), 49.5 (C-4), 48.6 (C-8), 41.2 (C-15), 38.2 (C-22), 36.5 (C-5), 34.5 (C-10), 33.4 (C-16), 31.6 (C-21), 19.9 (16-CH₃), 19.7 (C-12), 13.0 (C-11), 12.3 (18-CH₃).

Cheatoglobosin F_{ex} (**12**): Yellow solid. ESI-MS m/z 531 [M+H]⁺. ¹H NMR (CDCl₃, 400MHz) δ : 8.46 (1H, 1'-NH), 7.47 (1H, d, $J = 8.0\text{Hz}$, H-4'), 7.37 (1H, d, $J = 8.0\text{Hz}$, H-7'), 7.20 (1H, m, H-5'), 7.13 (1H, m, H-6'), 7.00 (1H, d, $J = 2.0\text{Hz}$, H-2'), 6.21 (1H, m, H-13), 6.13 (1H, d, $J = 9.2\text{Hz}$, H-17), 5.81 (1-NH), 5.37 (1H, m, H-14), 5.37, 5.13 (2H, m, H-12), 4.70 (1H, br, H-20), 3.92 (1H, d, $J = 10.4\text{Hz}$, H-7'), 3.79 (1H, br, -OH), 3.45 (1H, m, H-3), 2.90 (2H, H-10), 2.76, 2.63 (2H, m, H-22), 2.78 (1H, H-4), 2.58, 2.07 (2H, m, H-21), 2.42 (1H, m, H-5), 2.41, 2.04 (2H, m, H-15), 1.84 (3H, H-8, 18-CH₃), 1.03 (3H, d, $J = 6.4\text{Hz}$, H-11), 0.96 (3H, d, $J = 6.0\text{Hz}$, 16-CH₃). ¹³C NMR

(CDCl₃, 100MHz) δ : 208.2 (C-23), 203.9 (C-19), 174.3 (C-1), 149.5 (C-17), 148.5 (C-6), 136.8 (C-1'a), 135.9 (C-14), 134.9 (C-18), 128.9 (C-13), 127.5 (C-3'a), 123.6 (C-2'), 122.9 (C-5'), 120.4 (C-6'), 118.8 (C-12), 111.9 (C-7'), 111.2 (C-3'), 71.9 (C-20), 70.3 (C-7), 63.2 (C-9), 52.7 (C-3), 49.9 (C-8), 47.9 (C-4), 41.4 (C-15), 37.9 (C-22), 34.1 (C-5), 33.8 (C-16), 32.5 (C-10), 31.8 (C-21), 20.3 (18-CH₃), 14.3 (C-11), 12.6 (16-CH₃).

Penochalasin F (**13**): Needle crystal. ¹H NMR (DMSO-d₆, 500MHz) δ : 8.23 (1'-NH), 7.40 (1H, d, J = 7.7Hz, H-4'), 7.31 (1H, d, J = 7.9Hz, H-7'), 7.08 (2H, m, H-5', 6'), 6.89 (1H, d, J = 2.0Hz, H-2'), 6.03 (1H, dd, J = 9.9Hz, 15.2Hz, H-13), 5.91 (1H, 2-NH), 5.38 (1H, d, J = 8.8Hz, H-17), 5.13 (1H, m, H-14), 4.39 (1H, d, J = 3.4Hz, H-19), 4.05 (1H, m, H-3), 3.74 (1H, d, J = 3.2Hz, 19-OH), 3.68 (1H, d, J = 3.6Hz, 19-OH), 3.06, 2.59 (2H, m, H-22), 2.87 (2H, m, H-10), 2.74 (1H, d, J = 5.6Hz, H-7), 2.61 (1H, m, H-4), 2.47 (1H, m, H-16), 2.25 (1H, d, H-5), 2.17, 1.86 (2H, m, H-21), 2.16, 1.80 (2H, m, H-15), 1.88 (1H, H-8), 1.61 (3H, s, 18-CH₃), 1.33 (3H, s, H-12), 1.12 (3H, d, J = 7.2Hz, H-11), 0.95 (3H, d, J = 7.2Hz, 16-CH₃). ¹³C NMR (DMSO-d₆, 125MHz) δ : 211.8 (C-23), 207.9 (C-20), 174.9 (C-1), 140.3 (C-17), 136.7 (C-1'a), 134.3 (C-14), 131.5 (C-18), 128.4 (C-13), 127.5 (C-3'a), 124.0 (C-2'), 123.0 (C-5'),

120.7 (C-6'), 118.8 (C-4'), 112.1 (C-7'), 110.2 (C-3'), 82.7 (C-19), 64.31 (C-9), 62.1 (C-7), 57.5 (C-6), 52.9 (C-3), 49.9 (C-4), 49.7 (C-8), 42.1 (C-15), 36.9 (C-5), 36.7 (C-22), 34.4 (C-21), 34.3 (C-10), 32.6 (C-16), 20.0 (C-12), 13.4 (C-11), 11.0 (18-CH₃).

Penochalasin G (**14**): Needle crystal. ESI-MS m/z 537[M+Na]⁺. ¹H NMR (CDCl₃, 500MHz) δ : 8.11 (1'-NH), 7.41 (1H, d, J = 7.7Hz, H-4'), 7.31 (1H, d, J = 8.1Hz, H-7'), 7.14 (1H, m, H-5'), 7.07 (1H, m, H-6'), 6.95 (1H, d, J = 2.0Hz, H-2'), 5.94 (1H, dd, J = 15.2; 10.1Hz, H-13), 5.53 (1H, 2-NH), 5.45 (1H, d, J = 8.5Hz, H-17), 5.04 (1H, ddd, J = 14.7; 10.9; 3.3Hz, H-14), 4.43 (1H, d, J = 3.2Hz, H-19), 3.74 (1H, d, J = 3.2Hz, 19-OH), 3.36 (1H, m, H-3), 3.32, 2.60 (2H, m, H-22), 2.69, 2.55 (2H, m, H-10), 2.58 (1H, m, H-4), 2.55 (1H, m, H-5), 2.46 (1H, br, H-16), 2.35, 1.97 (2H, m, H-21), 2.22, 1.89 (2H, m, H-15), 2.17 (1H, m, H-8), 1.70 (3H, s, H-12), 1.34 (3H, s, 18-CH₃), 1.26 (3H, d, J = 7.3Hz, H-11), 0.92 (3H, d, J = 6.7Hz, 16-CH₃). ¹³C NMR (CDCl₃, 125MHz) δ : 212.0 (C-23), 209.0 (C-20), 174.7 (C-1), 140.7 (C-17), 140.6 (C-6), 136.7 (C-1'a), 132.1 (C-13), 131.3 (C-18), 127.5 (C-3'a), 126.3 (C-2'), 123.6 (C-14), 122.9 (C-6'), 120.5 (C-5'), 119.0 (C-4'), 111.9 (C-7'), 111.2 (C-3'), 82.5 (C-19), 67.1 (C-9), 54.1 (C-3), 51.9 (C-4), 48.4 (C-8), 42.0 (C-15), 37.3 (C-22), 35.4 (C-5),

34.6 (C-21), 34.5 (C-10), 32.7 (C-16), 21.5 (16-CH₃), 20.5 (C-12), 14.3 (C-11), 10.9 (18-CH₃).

Anti-phytopathogenic activity assay

Antifungal tests were performed as described by Park J.H. with some modification (Park **et al.** 2005). In the *vitro* antifungal test, PDB was used as incubation medium. 5 μ L of metabolite's solutions in dimethyl sulfoxide (DMSO) were added into 96-well sterilised microplates and their final concentrations ranged from 512 to 1 μ g/mL by using a twofold serial dilution method. 10 μ L spore suspension (10^5 spores/mL) of *Fusarium flocciferum* and mycelial suspensions (10^5 spores/mL) of the other fungi (*Phoma herbarum*, *Cladosporium oxysporum*, *Plectosphaerella cucumerina*, *Epicoccum nigrum*) were inoculated in each well. The wells contained fungi suspensions and DMSO were run as negative controls and the wells contained nystatin (Taicheng Pharmaceutical Co., Ltd, Guangdong, China) were introduced as the positive control. The plates contained test strains, and diluted compounds were incubated at 28°C (18-48 h), the minimum inhibitory concentrations of the antifungal metabolites that completely inhibited mycelial growth were defined as the MICs.

Acetylcholinesterase (AChE) inhibition assay

AChE inhibitory activities of the compounds were assayed by the spectrophotometric method developed by Ellman et al with slightly modification (Ellman **et al.** 1961). *S*-Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 5,5'-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), acetylcholinesterase derived from human erythrocytes were purchased from Sigma Chemical. Compounds were dissolved in DMSO. The reaction mixture (totally 200 μ L) containing phosphate buffer (pH 8.0), test compound (50 μ M), and acetyl cholinesterase (0.02 U/mL), was incubated for 20 min (37 $^{\circ}$ C). Then, the reaction was initiated by the addition of 40 μ L of solution containing DTNB (0.625mM) and acetylthiocholine iodide (0.625 mM) for AChE inhibitory activity assay, respectively. The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 seconds for one hour. Tacrine (TA) was used as positive control with final concentration of 0.333 μ M. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = $(E - S)/E \times 100$ (E is the activity of the enzyme without test compound, and S is the activity of enzyme with test compound).

96-Well Plate DPPH Method

The 96-Well plate DPPH method was performed as described by Routray et al. with some modification (Joshi et al. **2014**). The DPPH was dissolved in ethanol absolute then prepared to be stock solution at a concentration of 0.507 mM monthly and kept at 4 $^{\circ}$ C in dark. The 0.264 mM fresh DPPH working solution was made daily by further

diluting the stock solution in ethanol absolute for the test. The stock solutions of all compounds were prepared in DMSO at the concentration of 5.12 mg/mL. Ascorbic acid was used as positive control.

This 96-Well plate DPPH method was carried out using an infinite M200 PRO plate reader. Each well contained 5 μ L of sample solution, 95 μ L of ethanol and 100 μ L of 0.264 mM DPPH solution, a blank with only 5 μ L of DMSO and 195 μ L of ethanol and a control with the mixture of 100 μ L of ethanol and 100 μ L of 0.264 mM DPPH solution, the total volume of each well was 200 μ L and the final concentration of DPPH is 0.132 mM. The absorptions were determined at 517 nm. The % DPPH quenched was determined according to the equation:

$$\% \text{ DPPH quenched} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}})] \times 100$$

A 6 hour preliminary screening was employed to determine the standard type of the compounds (the final concentration of each sample solution was 128 μ g/mL). Then the EC₅₀ of suitable compounds were tested. EC₅₀ value is the effective concentration that could scavenge 50% of the DPPH radicals.

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Figure S1. HR-ESIMS of compound **1**

Figure S2. ^1H NMR of compound **1**

Figure S3. ^{13}C NMR of compound **1**

Figure S4. ^1H NMR of compound **2**

17-29 (DMSO) 477

Figure S5. ^{13}C NMR of compound **2**

17-26 (MeOD)

Figure S6. ^1H NMR of compound **3**

Figure S7. ^{13}C NMR of compound **3**

Figure S8. ^1H NMR of compound **4**

17-22 (DMSO) [450, 451, 453]

Figure S9. ^{13}C NMR of compound **4**

Figure S10. HR-ESIMS of compound **5**

17-13 (3) DMSO

Figure S11. ^1H NMR of compound **5**

17-13 (3) DMSO

Figure S12. ^{13}C NMR of compound **5**

Figure S13. ¹H NMR of compound **6**

Figure S14. ¹³C NMR of compound **6**

Figure S15. ¹H NMR of compound **7**

Figure S16. ¹³C NMR of compound **7**

Figure S19. ^{13}C NMR of compound **8**

Figure S20. HR-ESIMS of compound **9**

17-2 (CDCl₃)

Figure S21. ¹H NMR of compound **9**

Figure S22. ¹³C NMR of compound **9**

Figure S23. ^1H NMR of compound **10**

Figure S24. ^{13}C NMR of compound **10**

Figure S25. HR-ESIMS of compound **11**

17-7 (CDCl₃)

Figure S26. ¹H NMR of compound **11**

Figure S27. ¹³C NMR of compound **11**

Figure S28. HR-ESIMS of compound **12**

7.487
7.467
7.466
7.366
7.261
7.233
7.213
7.193
7.183
7.128
7.145
6.221
6.209
6.129
6.152
5.382
5.395
5.395
5.394
5.392
5.347
5.487
3.436
3.939
2.804
2.826
2.799
2.781
2.668
2.657
2.613
2.987
2.444
2.429
2.384
2.365
2.307
2.045
1.842
1.876
1.631
1.476
1.318
1.297
1.244
1.227
1.156
1.140
1.033
1.049
0.957

NAME 2201000170
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PROCNO 1
Date_ 20140917
Time 20.18
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PROBHD 5 mm PABBO BB
PULPROG zgpg30
TD 65536
SOLVENT DMSO-d6
NS 16
DS 2
SWH 8012.827 KHz
FIDRES 0.152764 Hz
AQ 4.0894966 sec
RG 120.65
DQ 82.400 usec
GB 6.50 usec
TE 299.2 K
DE 1.00000000 sec
TD0
----- CHANNEL f1 -----
SFO1 400.1527008 MHz
NUC1 1H
P1 10.40 usec
PL1 0.00 dB
SFO2 400.1500909 MHz
NUC2 1H
P2 0.00 dB
PL2 0.00 dB
GB 0.30 dB
PC 1.00

1.000
1.519
1.841
2.310
1.811
1.394
2.241
1.053
2.494
1.279
1.004
1.407
1.516
1.855
1.424
3.821
4.591
2.279
6.728
23.404
2.189
2.006
4.762
5.749

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

Figure S30. ^{13}C NMR of compound **12**

Figure S31. ¹H NMR of compound **13**

Figure S31. ¹³C NMR of compound **13**

Figure S32. HR-ESIMS of compound **14**

Figure S33. ¹H NMR of compound **14**

Figure S34. ^{13}C NMR of compound **14**