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Chaetosemins A-E, New Chromones Isolated from an Ascomycete *Chaetomium seminudum* and Their Biological Activities

He Li,[a] Jun-Mian Tian,[a] Hao-Yu Tang,[a] Shi-Yin Pan,[b] An-Ling Zhang,[a]* and Jin-Ming Gao[a]*

[a]Shaanxi Key Labotory of Natural Products & Chemical Biology, College of Science, Northwest A&F University, Yangling 712100, Shaanxi, China

[b]Xi'an No.1 Hospital, Shaanxi Institute of Ophthalmology, Xi'an 710002, Shaanxi,China

*Corresponding author:

Prof. An-Ling Zhang and Prof. Dr. Jin-Ming Gao. Tel.: +86-29-87092515

E-mail: jinminggao@nwsuaf.edu.cn

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ABSTRACT

Fifteen aromatic polyketide metabolites, including four new chromones, chaetosemins B-E (**2**-**5**), with compound **4** bearing a new skeleton, and two new natural products, chaetosemin A (**1**) and (+)-(*S*)-chaetoquadrin J (**14**), together with nine known ones (**6**-**13**, **15**), were isolated from the organic extract of a solid fermented culture of an Ascomycete filamentous fungus, *Chaetomium seminudum*. The structures of **1-5** were determined by spectroscopic analysis, and the absolute configuration of chaetosemin A (**1**) was confirmed by X-ray crystallography, whereas the stereochemistry of **1-4** and **14** was assigned by comparison of their specific optical rotations with reported data for structurally related compounds. All compounds were isolated for the first time from *Chaetomium seminudum*. Compounds **1** and **2** consisted of L-cysteine and D-cysteine unit, respectively. Most of them were evaluated for *in vitro* antifungal activities against some phytopathogenic fungi. Among them, chaetosemin B (**2**) was the most active against *Magnaporthe oryzae* and *Gibberella saubinettii* with MICs of 6.25 and 12.5 μM, respectively. In addition, chaetosemin C (**3**) showed antioxidant activity with 50.7 % DPPH free radical scavenging activity at 50 μM, and chaetoquadrin J (**14**) displayed weak inhibition of soluble epoxide hydrolase (sEH).

Keywords: *Chaetomium seminudum*; Chaetomiaceae; fungal chromone; eugenetin derivatives; plant pathogenic fungi

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Introduction

The genus *Chaetomium* (family Chaetomiaceae) comprises over 300 species.[1] Members of this filamentous fungal genus are distributed widelyin soil, air, plant debris and some of them arepathogenic to human.[2] Due to the diversity of species and of inhabiting-environments, *Chaetomium* spp. might conceive diverse biosynthetic gene clusters, which transform into various secondary metabolites to adapt to different ecological environments.[3,4]

Previous phytochemical investigations on the genus established the presence of numerous different classes of natural products, with over 200 compounds reported from about 30 species of this genus.[5] Most of these fungal metabolites exhibited antitumor, cytotoxic, antimalarial, enzyme inhibitory, antibiotic, and other activities.[5-11] Compared with its richness of species, more bioactive secondarys might be found in this member of fungi. Previous studies on *Chaetomium seminudum* led to the isolation of several chetomin derivatives with immunomodulatory activity.[12]

In continuation of our search for bioactive secondary metabolites produced by *Chaetomium* species,[13-16] we isolated and identified four new compounds (**2−5**) and eleven known compounds (**1**, **6−15**) from the organic extract of a solid-state fermented culture of the fungus. Herein, the isolation, structure determination, and biological activity of the metabolites are described.

Results and discussion

The solid-state fermented culture of *C. seminudum* was extracted with MeOH and then concentrated under reduced pressure to give a crude extract.The extractwas dissolved in H_2O and extracted with petroleum ether and EtOAc, successively. The petroleum ether- or EtOAc-solubleportion was subjected to repeated column chromatography over RP-18, silica gel, and Sephadex LH-20, leading to the isolation of four new compounds (**2-5**) and 11 known

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compounds (**1**, **6-15**) (Figure 1), and their structures were elucidated from analysis of their spectroscopic data.

Compound **1** was isolated as a pale yellow powder and its molecular formula $C_{15}H_{16}O_7S$ determined by HRESIMS, indicating eight degrees of unsaturation.The IR absorptions (1740 and 1665 cm^{-1}) and two quaternary carbon signals (δ_C176.2 and 183.9) in the ^{13}C NMR spectrum (Table 1) indicated the presence of carboxyl and ketone carbonyl groups in **1**. The UV spectrumshowed maximal absorption bonds at 290, 258, 251, 238, and 232 nm, suggesting that **1** was achromone derivative.[17] The ^{13}C NMR spectrumindicated the presence of 15 carbon signals, including two methyls, two methlenes, three methines, and eight quaternary carbons. The chemical shift of the phenolic hydroxy group (δ_H 12.81) suggested the presence of an intramolecular hydrogen bond between 5-OH and C-4 carbonyl groups. The NMR data of **1** were similar to those of eugenetin (**6**).[17] The main difference was that a methyl group at C-6 in **6** was replaced by a side chain containing sulfur (-$CH_2SCH_2CH(OH)COOH$) (δ_C 24.1, 37.5, 71.4, and 176.2) in **1**. The HMBC correlations of H-1′ to C-5, C-6, C-7, and C-3′, H-3′ to C-1′, C-4′, and C -5′, and H-4′ to C-3′ and C-5′ as well as a COSY correlation of H-3′/H-4′ suggested that the sulfur-containing side chain was located at C-6 (Figure 2). All these considerations allowed us to establish the planar structure of **1** as shown. Although the structure had been recorded previously in SciFinder, both its NMR data and stereochemistry were not reported. Accordingly, the complete assignments of all the protons and carbons were accomplished by HMBC, COSY, and HSQC experiments, as shown in Table 1. X-ray crystallographic analysis (Figure 3) further confirmed the structure and (*S*) absolute configuration of the only stereogenic center, C-4′ in **1.** Thus, compound **1** possesses a *S* configuration and named chaetosemin A.

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Compound **2** was obtained as a pale yellow powder and its molecular formula $C_{16}H_{18}O_7S$ determined by HRESIMS. The difference of 14 mass units between **2** and **1** indicated that **2** had one methylene unit more than **1**. It had very similar NMR and UV absorption data to those of **1** (Table 1), except for the presence of -OCH_3 (δ_H 3.76, s; δ_C 52.4) on the side chain. Further analysis of HMBC and COSY spectra corroborated the planar structure of **2** (Figure 2). The absolute configuration of **2** was determined as *R* by comparison of its optical rotation, $[\alpha]_D^{28}$ -6.3 (*c* 0.2, MeOH), since its sign was opposite to that of **1**, $[\alpha]_D^{28}$ + 9.3 (*c* 0.2, MeOH). Thus, the structure of **2** was established, and named chaetosemin B. Furthermore, considering the two compounds **1** and **2** with L-cysteine and D-cysteine residue, respectively, the specific optical rotation values of L-cysteine and D-cysteine were found to be $[\alpha]_D$ +8.3°, -6.4°, respectively, which are in full agreement with those for compounds **1** and **2.** Therefore, these data confirmed their stereochemistry as shown.

Compound **3** was isolated as a yellow powder and its molecular formula $C_{22}H_{24}O_7$ determined by HRESIMS. The ^{13}C NMR data (Table 2) showed the presence of 22 carbon signals, including four methyls, two methlenes, four methines, and 12 quaternary carbon signals. Detailed analysis of the ^{1}H and ^{13}CNMR data suggested that **3** was composed of two substructures: chromone and dihydroxyphenol ring. Connection of the two substructures was attained with the aid of HMBC correlations of H-9 to C-5, C-6, C-7, C-1′, C-2′, and C- 6′. The HMBC correlations of H-7′ to C-1′, C-5′, C-6′, C-8′, and C-9′, H-9′ to C-7′ and C-8′, and H-5′ to C-1′, C-3′, C-4′, and C-7′proved that a -$CH_2CH(OH)CH_3$ moiety was located at C-6′ (Figure 2), as confirmed by COSY correlations of H-7′/H-8′and H-8′/H-9′. The dihydroxyphenol ring part in **3** was quite similar to that of the known compound (*R*)-3-phenyl-propan-2-ol or (*S*)-orcinotriol.[18,19] The absolute configuration of **3** was determined by comparison of its optical

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rotation. The configuration of C-8′ in **3** was assigned as *R* since its optical rotation, $[\alpha]_D^{28}$-17.2 (*c* 0.1, MeOH) matched that of (*R*)-3-phenyl-propan-2-ol, $[\alpha]_D^{22}$-24.3 (*c* 0.1, $CHCl_3$),[18] but opposite to that of (*S*)-orcinotriol, $[\alpha]_D^{25}$+ 6.0 (*c*1.1, MeOH).[19] Thus, the structure of **3** was determined, and named chaetosemin C.

Compound **4** was isolated as a white powder and its molecular formula $C_{14}H_{16}O_4$ determined by HRESIMS. Likewise, the UV spectrum indicatd that **4** was also a chromone derivative.[17] The ^1H and ^{13}C NMR data (Table 2) showed the presence of 14 carbon signals, including three methyls, one methlene, three methines, and seven quaternary carbons. It possessed very similar ^1H and ^{13}C NMR data to those of 2-(2′-hydroxypropyl)-5-methyl-7-hydroxychromone.[20] These findings suggested the location of the methyl group at C-5 and of a 2-hydroxypropyl side chain at C-2. The structure of **4** differed from 2-(2′-hydroxypropyl)-5-methyl-7-hydroxychromoneonly in the location of an additional methyl group (δ_H2.19, s; δ_C 11.6) at C-6 (δ_C 124.4), as supported by HMBC correlations of CH_3-6 to C-5, C-6, and C-7 as well as a NOESY correlation of CH_3-6/CH_3-7. Key HMBC and COSY correlations confirmed the gross structure of **4** (Figure 2). To the best of our knowledge, this compound represents a new carbon skeleton of chromone. The stereoconfiguration of C-2′ in **4** was determined as *S* since the optical rotation of **4**, $[\alpha]_D^{20}$+58.6 (*c* 0.1, MeOH), agreed with that of 2-(2′-hydroxypropyl)-5-methyl-7-hydroxychromone,[20] $[\alpha]_D^{20}$+38.4 (*c* 0.89, MeOH).[20] Thus, the structure of **4** was determined, and it was named chaetosemin D.

Compound **5** was found to possess the molecular formula $C_{18}H_{22}O_{10}$ determined by HRESIMS. On the basis of 1D and 2D NMR data (Table 2, Figure 2) as well as comparison with eugenetin (**6**), **5** is a glycosylated derivative of **6**. One anomeric proton at δ_H 4.79 (d, *J*= 7.53 Hz, H-1′) in the ^1H NMR spectrumas well as one anomeric carbon at δ_C 103.9 in the ^{13}C NMR

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spectrum indicated the existence of one sugar moiety. Acid hydrolysis afforded D-glucosebased on GC-MS analysis.[21] The anomeric proton coupling constant (J = 7.5 Hz) established a β-glucose configuration.[22] In addition, HMBC correlations were used to assign the C-8 position of the sugar unit with correlations of H-1′(δ_H 4.79)/C-8 (δ_C 128.9) (Figure 2). From these spectroscopic data analysis, the structure was assigned as 8-(β-D-glucopyranoxyl)-5-hydroxy-7-methoxy-2,6-dimethylchromone, and it was named chaetosemin E.

(+)-*S*-Chaetoquadrin J (**14**) had identical ^{1}H and ^{13}C NMR data to those of chaetoquadrin J.[23] It showed a positive value of the optical rotation, $[\alpha]_D^{21}$+33.9 (*c* 0.1, $CHCl_3$), opposite to that reported previously, $[\alpha]_D^{20}$-30.9 (*c* 0.2, $CHCl_3$).[22] The absolute configuration of C-3′ in **14** was assigned as *S* from the positive optical rotation, similar to that of known related compounds.[24] Thus, compound **14** is a new natural product with *S*-configuration.

The structures of the known compounds were identified as eugenetin (**6**),[25] 6-methoxymethyleugenin (**7**),[26] 6-hydroxymethyleugenin (**8**),[26] eugenitol (**9**),[25] isoeugenitin (**10**),[27] chaetoquadrins D (**11**),[28] G (**12**), H (**13**),[23] and chaetoaurin (**15**)[17] by comparison of their spectroscopic data with published values and were described for the first time in *C. seminudum*.

In nature, chiral natural products are usually produced in optically pure form; however, sometimes, there are examples that these enantiomeric natural products could be obtained as mixture with 1:1 or less than 1:1 (two eantiomers are not with the same quantity), or obtained only one of the enantiomers.[29] For example, Kato et al. described the isolation of an enantiomeric mixture of 6-epi-stephacidin A enriched with the (-)-isomer from the fungus *Aspergillus amoenus*.[30] Interestingly, Asai et al. reported only esterified one of a pair of the isariotin-type enantiomers in the fungus entomopathogenic, *Gibellula formosana*.[31] In the

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current work, we can not obtain the *R* and *S* mixture (enantiomers) of **1** and **2**, but only esterified one of the enantiomers. As a result, compound **2** could be not an artifact and should be actually generated by fungal fermentation. The formation of this methyl ester could be responsible for the esterified enzyme found in *Chaetomium seminudum.*

Compounds **1-11**, and **15** were evaluated for their antifungal and antioxidant activities.[32,33] In the antifungal assay against five plant pathogens (*B. cinerea*, *A. solani*, *C. gloeosporioides*, *G. saubinettii*, and *M. oryzae*). As shown in Table 3, only **2** exhibited inhibitory activity against *B. cinerea*, *M. oryzae*, and *G. saubinettii*, with MICs of 50, 6.25, and 12.5 μM, respectively, which were similar to or weaker than carbendazim (MICs of 6.25 μM) as a positive control, but inactive to *A. solani* and *C. gloeosporioides*. In contrast, no activity for **1** was observed toward the same pathogens (MIC > 100 μM). This indicated that the –$COOCH_3$ group seems to be favorable for antifungal activity. Moreover, **3** showed moderate anti-oxidant activity with 50.7 % DPPH free radical scavenging activity at 50 μM, while the other compounds were inactive, suggesting that the chromone moiety could not increase anti-oxidant activity; however, the presence of the dihydroxyphenolring in **3** was able to enhance the activity.

sEH inhibitors have been shown to possess antihypertensive and anti-inflammatory effects and to protect the brain, heart and kidney from damage.[34] Compounds **1**, **2**, **6-10**, and **14** were tested for their sEH inhibitory activity,[35] and only **14** decreased she activity to 44.9% at 100 μM, with an IC_{50} value of 63.0 ± 0.8 μM, while the IC_{50} of AUDA, the positive sEH inhibitor, was 10.9 ± 1.2 nM. The results indicated that **14** displayed weak sEH inhibitory activity.

In conclusion, in this study, the solid-state fermented *C. seminudum* produced new polyketide-derived chromone derivatives, chaetosemins B-E (**2-5**), along with two new natural products, chaetosemin A (**1**) and (+)-*S*-chaetoquadrin J (**14**). Our findings would enrich chemical

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context of the genus *Chaetomium* and to expand the chemistry of chromones. Among the isolated ones, compound (**2**) is the most active antifungal agent against two agricultural pathogens, *M. oryzae* and *G. saubinettii*, and chaetosemin C (**3**) showed anti-oxidant activity.

Experimental

General Experimental Procedures. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Qingdao Marine Chemical Ltd). Column chromatography(CC) was performed on MCI gel (75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan), 200−300 mesh silica gel (Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Pharmacia), and RP-18 (Merck). Fractions were monitored by TLC, and spots were visualized by spraying with 5% H_2SO_4 in ethanol, followed by heating. All other chemicals used in this study were of analytical grade. Optical rotations were measured on an Autopol III automatic polarimeter (Rudolph Research Analytical).UV spectra were measured by a Thermo Scientific Evolution-300 UV–visible spectrophotometer.IR spectra were run on a Bruker TENSOR27 spectrophotometer. NMR experiments were carried out on a BrukerAvance III 500or 400 spectrometer, with tetramethylsilane (TMS) as internal standard. Electrospray ionization mass spectrometry (ESI-MS) was obtained on a Bruker Esquire 6000 instrument, and high-resolution ESI-MS was recorded on a Thermo Fisher Scientific Q-TOF mass spectrometer. GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS.

Strain and Cultivation. The strain was purchasedfrom Shaanxi Institute of Microbiology. It was deposited at College of Science, Northwest A&F University. This fungus was cultivated on sterilized moistened rice in Roux flasks (100 g/flask ×200) at 25 °C for 28 days to give yellowish-green moldy rice.

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Extraction and Isolation. The cultures were ultrasonically extracted four times with MeOH, and the solvent was removed *in vacuo*to give a crude extract, which was suspended in H_2O and partitioned successively with petroleum ether (PE) and ethyl acetate (EtOAc). The PE extract (8.5 g) was submitted to CC over silica gel eluting with gradient PE/EtOAc (10:1 to 1:1) to give six subfractions A1-A6 based on TLC analysis. Subfraction A1 was subjected to CC on Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and further purified by preparative TLC (PE/EtOAc, 5:1) to afford compound **10** (6 mg). Compound **6** (30 mg) was recrystallized from subfraction A2 in $CHCl_3$. Subfraction A3 was separated using a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) followed by purification using preparative TLC (PE/EtOAc, 2:1) to give compound **7** (15 mg). Subfraction A4 was purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel ($CHCl_3$/acetone, 49:1) to give **8** (15 mg); subfraction A5 was also purified using the same isolation procedure as **8**, affording compounds **12** (5 mg), **13** (5 mg) and **15** (10 mg). Subfraction A6 was subjected to CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel ($CHCl_3$/acetone, 19:1) to obtain **9** (12 mg).

The EtOAc extract (40.0 g) was separated usinga silica gel column using $CHCl_3$, $CHCl_3$-CH_3OH (100:1, 50:1, 20:1, and 10:1) as elutent to give fractions A-E. Fraction A (12.8 g) was chromatographed over silica gel using gradient PE/EtOAc (10:1 to 1:1) to yield six subfractions A1-A6. Subfraction A6 was subjected to CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and then silica gel (PE/acetone, 3:1), and further purified by preparative TLC ($CHCl_3$/acetone, 19:1) to yield compound **14** (13 mg). Fraction B (6.9 g) was separated by a MCI column using MeOH-H_2O (10:90-100:0) to provide six subfractions B1-B6. Subfraction B3 was purified by CC on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$/acetone/HCOOH, 9:1:0.1) followed by purification using preparative TLC ($CHCl_3$/acetone/HCOOH, 7:3:0.1) to give

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compounds **1** (30 mg) and **11** (20 mg). Subfraction B4 was chromatographed over Sephadex LH-20 (MeOH), silica gel (PE/acetone, 7:3; $CHCl_3$/MeOH, 100:1), and preparative TLC ($CHCl_3$/MeOH, 19:1) to give compounds **2** (15 mg) and **3** (5 mg). Fraction D (4.28 g) was fractionated on a MCI column with MeOH-H_2O (10:90-100:0) to provide six subfractions D1-D6. Subfraction D4 was submitted to CC on Sephadex LH-20 (MeOH), silica gel (PE/acetone, 7:3) and further purified preparative TLC ($CHCl_3$/MeOH, 9:1) to give compound **4** (6 mg). Fraction E (4.0 g) was subjected to MCI CC using MeOH-H_2O (10:90-100:0) to provide six subfractions E1-E6. SubfractionE3 was rechromatographed by CC on Sephadex LH-20 (MeOH), silica gel ($CHCl_3$/MeOH, 9:1) and preparative TLC ($CHCl_3$/MeOH, 9:1) to provide **5** (12 mg).

Chaetosemin A (**1**): pale yellow powder; $[\alpha]_D^{28}$ + 9.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max}(logε) 290(4.18), 258 (4.64), 251 (4.64), 240(4.61), 231 (4.61) nm; IR (KBr) ν 3550, 3479, 3366, 2949, 1740, 1665, 1619,1588, 1498, 1445, 1406, 1392, 1343, 1308, 1262, 1084 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive ESIMS *m/z* 341.1 $[M+H]^+$; HRMSESI *m/z* 341.0668 $[M+H]^+$ (calcd. for $C_{15}H_{16}O_7S$, 341.0690).

Chaetosemin B (**2**): white powder; $[\alpha]_D^{28}$ −6.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max}(logε) 290(3.80), 258 (4.31), 251 (4.24), 238(4.23), 232 (4.24) nm; IR (KBr) ν 3424, 3234, 2997, 2952, 2850, 1744, 1664, 1634,1585, 1495, 1449, 1412, 1386, 1346, 1310, 1104, 1089 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive ESIMS *m/z* 354.73 $[M+H]^+$; HRMSESI *m/z*355.0821 $[M+H]^+$ (calcd. for $C_{16}H_{18}O_7S$, 355.0846).

Chaetosemin C (**3**): yellow powder; $[\alpha]_D^{28}$ −17.2 (*c*0.1, MeOH); UV (MeOH) λ_{max}(logε) 285 (3.72), 258 (3.99), 206 (4.47) nm; IR (KBr) ν 3425,2962, 2923, 2853, 1658, 1620,1575, 1494,

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1451, 1417, 1383, 1347, 1261, 1120 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; negative ESIMS *m/z* 399.42 $[M-H]^{-}$; HRMSESI *m/z* 399.1415 $[M-H]^{-}$ (calcd. for $C_{22}H_{24}O_7$, 399.1449).

Chaetosemin D (**4**): white powder; $[\alpha]_D^{20}$+ 58.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max}(logε) 293 (4.15), 252 (4.11), 241(3.99) nm; IR (KBr) ν 3447, 3425, 2967, 2925, 2853, 1664, 1613,1580, 1563, 1460, 1377, 1270, 1180 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 3; negative ESI-MS *m/z*247.19 $[M-H]^{-}$; HRMSESI *m/z* 247.0968 $[M-H]^{-}$ (calcd. for $C_{14}H_{16}O_4$, 247.0976).

Chaetosemin E (**5**): yellow powder; $[\alpha]_D^{20}$+ 87.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max}(logε) 277(4.15), 255 (4.24), 241 (4.28) nm; IR (KBr) ν 3437, 2919, 2850, 1657, 1618, 1475, 1450, 1409, 1385, 1367, 1266, 1108, 1093, 1064, 1008 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 4; positive ESIMS *m/z* 399.05 $[M+H]^{+}$; HRMSESI *m/z* 399.1286 $[M+H]^{+}$ (calcd. for $C_{18}H_{22}O_{10}$, 399.1302).

(+)-*S*-chaetoquadrin J (**14**): white powder; $[\alpha]_D^{21}$ +33.9 (*c* 0.1, $CHCl_3$); ^{1}HNMR (400 MHz, C_5D_5N) δ 6.46 (1H, s, H-8), 6.08 (1H, s, H-3), 4.56 (1H, m, H-3′), 3.97 (2H, s, H-9), 3.79 (3H, s, 7-OCH_3), 3.45 (1H, dd, J = 16.4, 3.0 Hz, H-4′a), 2.67 (1H, dd, J = 16.4, 11.8 Hz, H-4′b), 2.21 (1H, s, 2-CH_3), 2.20 (3H, s, 7′-CH_3), 1.39 (3H, d, J = 6.3 Hz, 3′-CH_3); ^{13}CNMR (100 MHz, C_5D_5N) δ 182.92 (C-4), 171.62 (C-1′), 168.10 (C-2), 163.69 (C-7), 161.49 (C-8′), 161.02 (C-6′), 158.24 (C-5), 157.19 (C-8a), 137.62 (C-4a′), 116.07 (C-5′), 111.09 (C-7′), 110.12 (C-6), 108.80 (C-3), 105.08 (C-4a), 101.01 (C-8a′), 90.90 (C-8), 75.59 (C-3′), 56.18 (7-OCH_3), 32.36 (C-4′), 20.77 (3′-CH_3), 19.82 (2-CH_3), 19.39 (C-9), 8.49 (7′-CH_3); negative ESI-MS *m/z* 425.42 $[M-H]^{-}$.

X-ray Crystallographic Analysis Data of Chaetosemin A (1): orthorhombic crystal of $C_{15}H_{16}O_7S{\cdot}H_2O$, M = 358.35, space group P2(1)2(1)2(1), a = 4.5675(5) Å, α = 90°; b = 7.6532(8) Å, β = 90°; c = 45.491(3) Å, γ = 90°, V =1590.2(3) $Å^3$, Z = 4, D_{calcd} = 1.497 mg/m^3, crystal size 0.42×0.30×0.28 mm, Mo Kα (λ=0.71073 Å), F(000)=752, T=298(2) K, Flack parameter = 0.04 (15). The final R values were R_1 = 0.0525, and wR_2 = 0.1052, for 7980 observed reflections (θ =

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2.81° to 25.01°) [I > 2σ(I)]. The single-crystal X-raydiffraction data of **1** were collected with a Bruker Smart-1000CCD area detector diffractometer with a rotating anode source (Mo Kα radiation, λ= 0.71073 Å) at 273(2) K. Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 952617. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 01223 336033 or email: deposit@ccdc.cam.ac.uk).

Acid Hydrolysis and Sugar Analysis. The process to identify D and L sugars by GCMS was described by our previously reported protocol.[21] Chaetosemin E (**5**) (2 mg) was hydrolyzed by using 1 M HCl (0.4 mL) at 100 °C for 2 h under argon and extracted with EtOAc (2 ×2 mL).The aqueous layer was neutralized with Amberlite IR400. After drying under reduced pressure, the residue was dissolved in pyridine (0.4 mL), and L-cysteine ethyl ester hydrochloride (2.0 mg) was added to the solution. The mixture was stirred at 60 °C for 1 h; then 0.3 mLof trimethylchlorosilane was added, followed by heating at 60 °C for 30 min. The reaction mixture was partitioned with *n*-hexane and distilled H_2O, and the *n*-hexane layer was analyzed by GCMS. The conditions of GCMS were as follows: capillary column, EQUI-TY™-1 (30 m × 0.25 mm × 0.25 mm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier, N_2 gas; detection in EI mode, ionization potential, 70 eV; ion-source temperature, 280 °C. D-Glucose was confirmed by comparison of the retention time of its derivative with those of L-glucose, D-glucose derivatives prepared in the same manner, showing the respective retention times of 11.21, 10.68 min.

Antifungal Bioassays.[32] The test phytopathogenic fungi used in this study were *Botrytis cinerea*, *Alternariasolani*, *Colletotrichum gloeosporioides*, *Gibberella saubinettii*, and

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Magnaportheoryzae. All the fungi were isolated from infected plant organs at the Northwest A&F University.

Antifungal activity was assessed by the microbroth dilution method in 96-well culture plates using a potato dextrose (PD) medium. The test compounds were made up to 4000μmol/L in DMSO. One commercial fungicide, carbendazim (Aladdin chemistry Co. Ltd.), was used as positive control, and the solution of equalconcentration of DMSO was used as negative control. The tested fungi were incubated in the PD medium for 18 h at 28 ± 0.5 °C at 150 rpm, and spores of different microorganism concentrations were diluted to approximately 1×10^6 CFU with PD medium. Test compounds (10 μL) were added to 96-well microplates, and 90 μL ofPD medium was added. Serial dilutions were made in the 96-well round-bottom sterile plates in triplicate in 50 μL of PD medium, and then 50 μL of the fungal suspension was added. After incubation for 48h at 28 ± 0.5°C, minimum inhibitory concentration (MIC) was taken as the lowest concentration of the test compounds in the wells of the 96-well plate in which no microbial growth could be observed.

Antioxidant Assays. DPPH free radical scavenging activity was measured according to our reported method.[33]

sEH Inhibitory Activity Assays.[35] sEH inhibitory activity was determined using a hydrolysis reaction of PHOME in the presence of the sEH enzyme. Briefly, 130 μL of sEH (62.5 ng/mL) in 25 mM Bis-Tris buffer (including 0.1% bovine serum albumin, pH 7.0) and 20 μL of ligands diluted in MeOH were mixed in 96 well plate. 50 μL of PHOME (40 μM) in buffer was added as substrate. After starting the sEH reaction at 37 °C, products were measured using a Geniosmicroplate reader (Tecan, Mannedorf, Switzerland) during an hour at excitation and

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emission wavelengths of 320 and 465 nm, respectively. sEH inhibitory activity for each sample was calculated as follows:

sEH inhibitory activity (%) = 100-[(S_{60}-S_0/C_{60}-C_0] × 100

whereC_{60} and S_{60} were the fluorescence of control and inhibitor after 60 min, S_0 and C_0 is the fluorescence of control and inhibitor at zero min.

Supporting Information

1D and 2D NMR, and IR spectra of compounds **1-5** and crystal data of**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes and references

1 X. W. Wang, Systematic studies on *Chaetomium* Kunzeand its morphologically simialr taxa from China, Chinese Academy of Sciences, 2005.

2 D. A. Sutton and A. W. Fothergill, *Guide to Clinically Significant Fungi, 1st ed.*, Williams & Wilkins Press, Baltimore, 1998.

3 A. C. Leeder, G. J. Palma and N. L. Glass, *Nat. Rev. Microbiol.*, 2011, **9**, 440−451.

4 T. Asai, T. Yamamoto, N. Shirata, T. Taniguchi, K. Monde, I. Fujii, K. Gomi and Y. Oshima, *Org. Lett.*, 2013, **15**, 3346–3349.

RSC Advances Accepted Manuscript

5 Q. Zhang, H. Q. Li, S. C. Zong, J. M. Gao and A. L. Zhang, *Mini. Rev. Med. Chem.*, 2012, **12**, 127−148.

6 A. Abdel-Lateff, *Tetrahedron Lett.*, 2008, **49**, 6398−6400.

7 C. M. Cui, X. M. Li, C. S. Li, P. Proksch and B. G. Wang, *J. Nat. Prod.*, 2010, **73**, 729−733.

8 G. Ding, Y. C. Song, J. R. Chen, C. Xu, H. M. Ge, X. T. Wang and R. X. Tan, *J. Nat. Prod.*, 2006, **69**, 302−304.

9 H. Oikawa, Y. Murakami and A. Ichihara, *Biosci.Biotech.Biochem.*, 1993, **57**, 628−631.

10 Y. Muroga, T. Yamada, A. Numata and R. Tanaka, *Tetrahedron*, 2009, **65**, 7580−7586.

11 S. Thohinung, S. Kanokmedhakul, K. Kanokmedhakul, V. Kukongviriyapan, O. Tusskorn and K. Soytong, *Arch. Pharm. Res.*, 2010, **33**, 1135−1141.

12 H. Fujimoto, M. Sumino, E. Okuyama and M. Ishibashi, *J. Nat. Prod.*, 2004, **67**, 98−102.

13 (a) J. M. Gao, S. X. Yang, J. C. Qin, *Chem. Rev.*, 2013, **113**, 4755-4811; (b) J. C. Qin, Y. M. Zhang, J. M. Gao, M. S. Bai, S. X. Yang, H. Laatsch and A. L. Zhang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1572−1574.

14 H. Li, J. Xiao, Y. Q. Gao, J. J. Tang, A. L. Zhang and J. M. Gao, *J. Agric. Food.Chem.*, 2014, **62**, 3734–3741.

15 H. Q. Li, X. J. Li, Y. L. Wang, Q. Zhang, A. L. Zhang, J. M. Gao and X. C. Zhang, *Biochem. Syst. Ecol.*, 2011, **39**, 876−879.

16 M. Xue, Q. Zhang, J. M. Gao, H. Li, J. M. Tian and G. Pescitelli, *Chirality*, 2012, **24**, 668−674.

17 L. M. Li, Q. Zou and G. Y. Li, *Chin. Chem. Lett.*, 2010, **21**, 1203−1205.

18 Y. Nakamura, H. Egami, K. Matsumoto, T. Uchida and T. Katsuki, *Tetrahedron*, 2007, **63**, 6383−6387.

RSC Advances Accepted Manuscript

19 H. Shigemori, M. Tenma, K. Shimazaki and J. Kobayashi, *J. Nat. Prod.*, 1998, **61**, 696−698.

20 Y. Kashiwada, G. I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1984, **32**, 3493−3500.

21 C. L. Zhang, J.M. Gao and W. Zhu, *Phytochem. Lett.* 2012, **5**, 49–52.

22 W. Q. Chen, Z. J. Song and H. H. Xu, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5819−5822.

23 H. Fujimoto, M. Nozawa, E. Okuyama and M. Ishibashi, *Chem. Pharm. Bull.*, 2003, **51**, 247−251.

24 M.J. Cheng, P.H. Yang, M.D. Wu, I.S. Chen, M.T. Hsieh, Y.L. Chen and G.F. Yuan, *Helv. Chim. Acta*, 2011, **94**, 1638−1650.

25 H. Foxand S. Huneck, *Phytochemistry*, 1969, **8**, 1301−1304.

26 (a) Y. Feng, J. W. Blunt, A. L. J. Coleand M. H. G. Munro, *J. Nat. Prod.*, 2002, **65**, 1682−1682. (b) X. J. Li, J. M. Gao, H. Chen, A. L. Zhang and M. Tang, *Eur. J. Plant. Pathol.*, 2012, **134**, 239−247.

27 W. Y. Tsui and G. D. Brown, *Phytochemistry*, 1996, **43**, 871−876.

28 F. Haruhiro, N. Masato. O. Emiand and I. Masami, *Chem. Pharm. Bull.*, 2002, **50**, 330−336.

29 J. M. Finefield, D. H. Sherman, M. Kreitman and R. M. Williams, *Angew. Chem. Int. Ed. Engl.*, 2012, **51**, 4802-4836.

30 H. Kato, T. Nakahara, K. Sugimoto, K. Matsuo, I. Kagiyama, J. C. Frisvad, D. H. Sherman, R. M. Williams and S. Tsukamoto, *Org. Lett.*, 2015, **17**, 700−703.

31 T. Asai, Y.-M. Chung, H. Sakurai, T. Ozeki, F.-R. Chang, Y.-C. Wu, K. Yamashita and Y. Oshima, *Tetrahedron*, 2012, **68**, 5817-5823.

32 C. L. Feng and Y. M. Ma, *J. Appl. Environ. Biol.*, 2010, **16**, 76−78.

RSC Advances Accepted Manuscript

33 C. D. Zheng, G. Li, H. Q. Li, X. J. Xu, J. M. Gao and A. L. Zhang, *Nat. Prod. Commun.*, 2010, **5**, 1759−1765.

34 J. D. Imig and B. D. Hammock, *Nat. Rev. Drug Discov.*, 2009, **7**, 894−805.

35 M.-M. Bai, W. Shi, J.-M.Tian, M.Lei, Y. H. Kim, Y. N.Sun, J. H. Kim and J.-M.Gao, *J. Agric. Food Chem.*, 2015, **63**, 2198-2205.

RSC Advances Accepted Manuscript

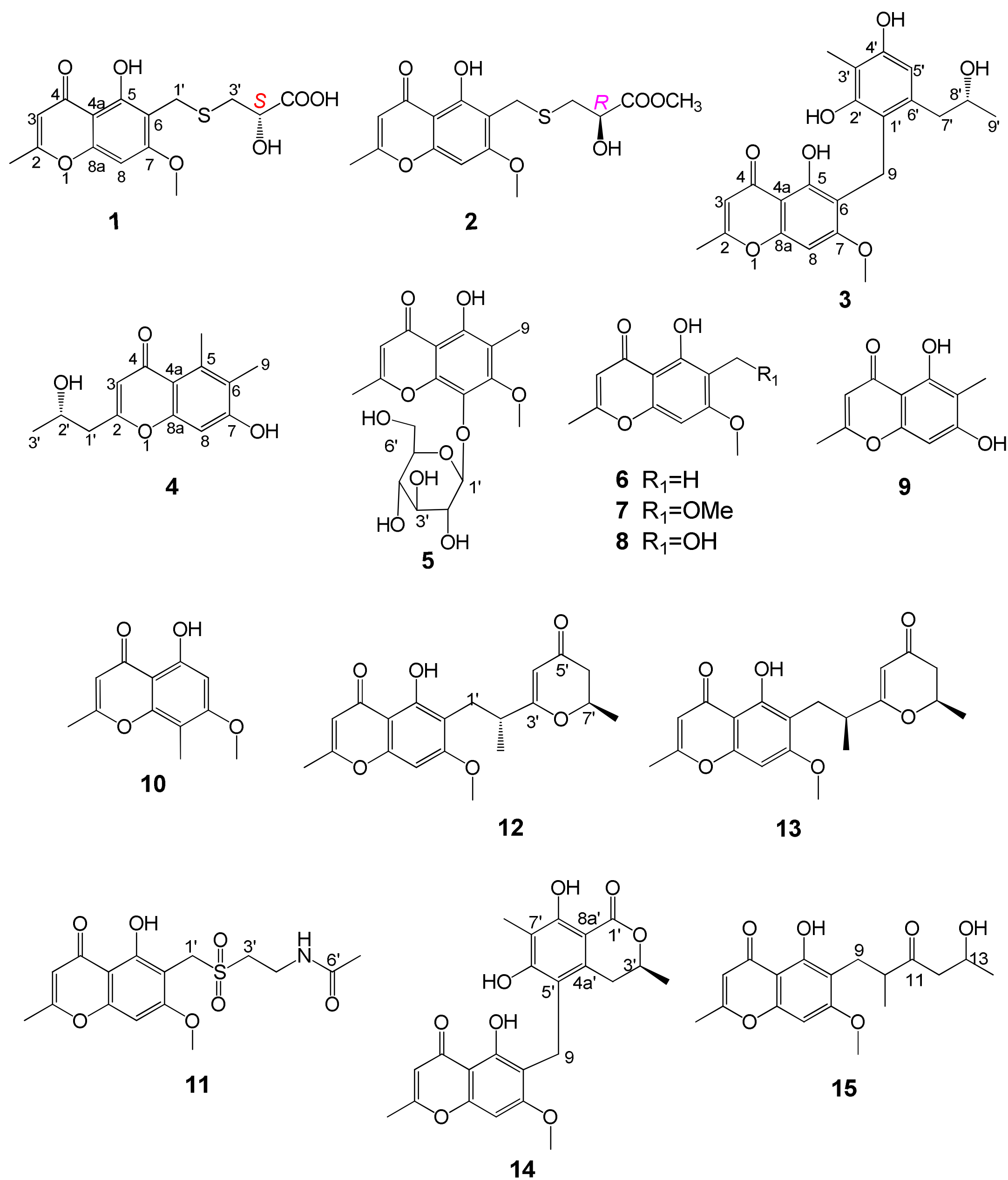

Figure 1. Structures of metabolites **1-15** isolated from *C. seminudum*

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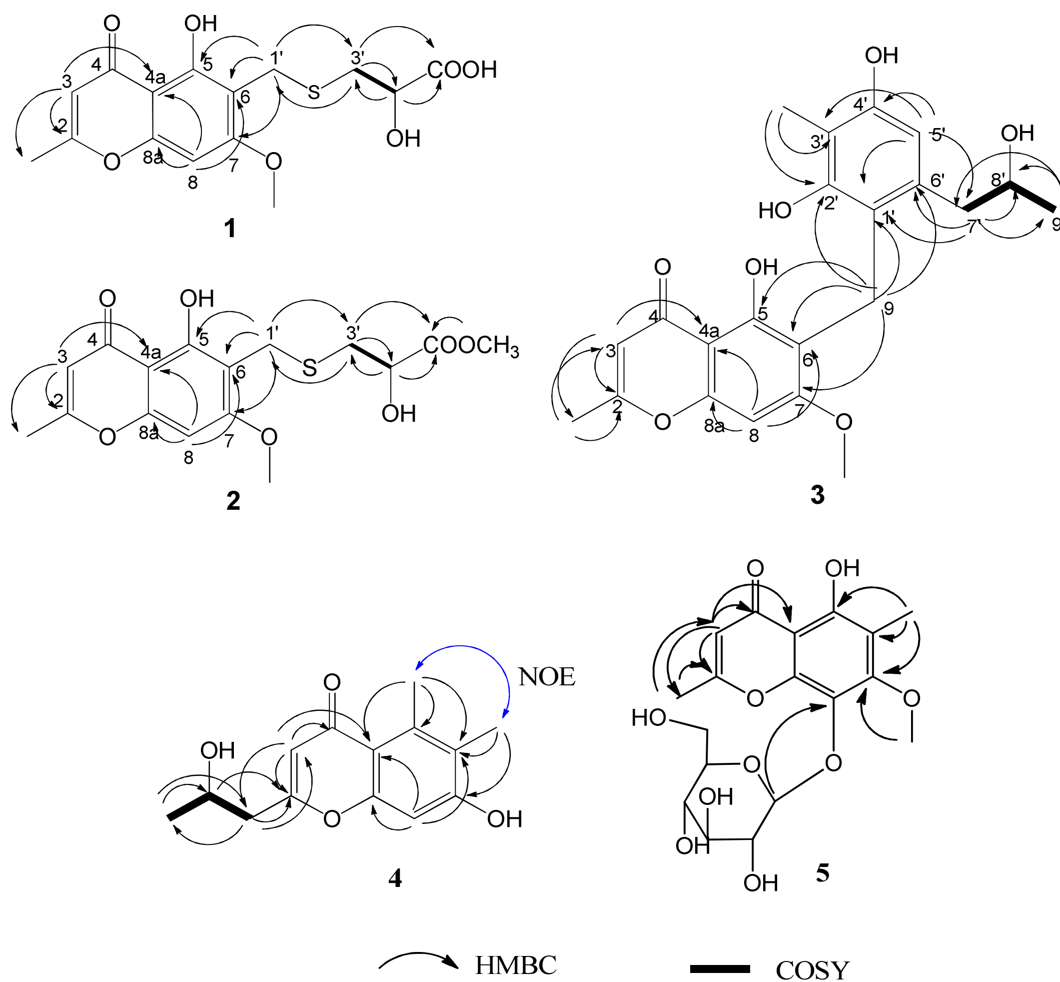

Figure 2.Key HMBC, COSY, and NOEcorrelations of **1**-**5**.

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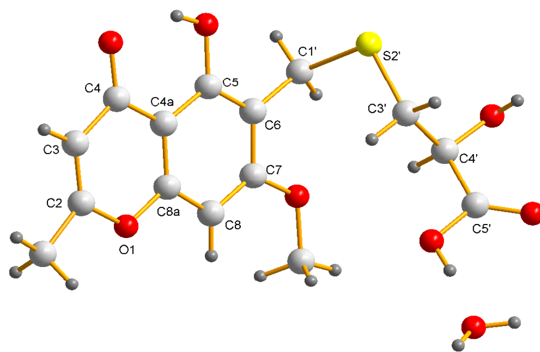

Figure 3. ORTEP diagram of compound **1**.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **1** and **2**

position	**1**[a] (MeOH-d_4)		**2**[a] ($CDCl_3$)	
	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)
2	169.4		166.7	
3	109.2	6.07 s	108.9	6.03 s
4	183.9		182.3	
4a	105.6		104.9	
5	159.3		158.7	
6	111.6		109.8	
7	164.5		162.8	
8	91.2	6.51 s	89.8	6.36 s
8a	158.6		157.2	
1′	24.1	3.79, d (12.9)	23.5	3.85, d (12.9)
3′	37.5	3.01, dd (13.6, 3.4) 2.79, dd (13.6, 7.4)	36.6	3.04, dd (13.6, 3.4) 2.85, dd (13.6, 7.4)
4′	71.4	4.30, dd (7.4, 3.4)	70.2	4.44, dd (7.4, 3.4)
5′	176.2		173.4	
7′			52.4	3.76, s
2-CH_3	20.4	2.36, s	20.4	2.34, s
7-OCH_3	56.8	3.91, s	56.1	3.90, s
5-OH		12.81, s		13.06, s

[a]Compounds**1** and **2** were measured at 400 MHz; assignments were confirmed by HSQC, HMBC, and COSY spectroscopy.

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Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **3**, **4**, and **5**

position	**3**[a] ($CDCl_3$)		**4**[a] (MeOH-d_4)		**5**[b] (DMSO-d_6)	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
2	167.5		166.3		168.5	
3	107.9	6.11, s	112.7	6.06, s	107.9	6.25, s
4	182.5		182.4		182.4	
4a	104.1		115.7		105.8	
5	156.4		140.9		153.6	
6	111.1		124.4		112.7	
7	162.9		161.4		156.7	
8	90.1	6.50, s	100.6	6.70, s	128.9	
8a	156.4		159.3		148.2	
9	18.6	3.90, overlapped	11.3	2.19, s	8.0	2.04, s
1′	114.8		44.1	2.69, m	103.9	4.79, d (7.5)
2′	153.1		66.9	4.20, dd (13.3, 5.8)	74.0	3.30, m
3′	109.4		23.5	1.29, d (6.3)	76.4	3.23, m
4′	153.7				69.9	3.15, m
5′	108.9	6.28, s			77.2	3.08, m
6′	135.5				61.0	3.40, d (11.4) 3.59, d (11.5)
7′	42.1	2.99, m				
8′	68.3	3.84, overlapped				
9′	21.7	1.19, d (6.1)				
2-CH_3	19.5	2.40, s			20.0	2.39, s
5-CH_3			17.6	2.76, s		
7-OCH_3	55.2	3.89, s			61.2	3.95, s
3′-CH_3	7.7	2.05, s				

[a]Compounds**3** and **4** were measured at 400 MHz; [b]compound**5** was measured at 500 MHz; assignments were confirmed by HSQC, HMBC, and COSY spectroscopy.

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Table 3. Inhibition of compounds on eight phytopathogenic fungi

compd.	phytopathogenicfungi（MIC: μM）				
	B. c.	*A. s.*	*M. o.*	*C. g.*	*G. s.*
1	200	200	200	>200	100
2	50	100	6.25	>200	12.5
3	>200	>200	>200	>200	>100
4	200	200	200	>200	100
5	200	200	200	>200	200
6	>200	>200	100	>200	100
7	50	200	100	>200	100
8	200	200	100	>200	100
9	50	200	100	>200	100
10	200	200	100	>200	100
11	>200	>200	>200	>200	200
14	>200	>200	>200	>200	>200
carbendazim	12.5	12.5	6.25	6.25	6.25

B.c., *Botrytis cinerea*; *A.s.*, *Alternaria solani*; *M.O.*, *Magnaporthe oryzae*; *C.g.*, *Colletotrichum gloeosporioides*; *G.s.*, *Gibberella saubinettii*.

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