JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Erinacerins C−L, Isoindolin-1-ones with α-Glucosidase Inhibitory Activity from Cultures of the Medicinal Mushroom *Hericium erinaceus*

Kai Wang,[†,‡] Li Bao,[†] Qiuyue Qi,[†,‡] Feng Zhao,[§] Ke Ma,[†] Yunfei Pei,[†] and Hongwei Liu*[,†]

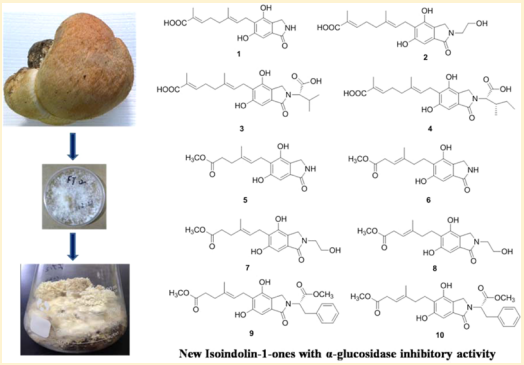
[†]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichenxi Road, Chaoyang District, Beijing 100101, People's Republic of China

[‡]University of Chinese Academy of Sciences, Beijing, 100049, People's Republic of China

[§]School of Pharmacy, Yantai University, No. 32 Qingquan Road, Laishan District, Yantai, 264005, People's Republic of China

Supporting Information

ABSTRACT: The well-known edible and medicinal mushroom *Hericium erinaceus* produces various bioactive secondary metabolites. Ten new isoindolin-1-ones, named erinacerins C−L (**1**−**10**), together with (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxy-6-methoxy-2-phenethylisoindolin-1-one (**11**) were isolated from the solid culture of *H. erinaceus*. The structures of new metabolites were established by spectroscopic methods. The absolute configurations of **3**, **4**, **9**, and **10** were assigned by comparing their specific rotations with those of related phthalimidines (**13**−**20**). Compounds **5** and **6**, **7** and **8**, and **9** and **10** are double-bond positional isomers. In a α-glucosidase inhibition assay, compounds **2**−**11** showed inhibitory activity with IC_{50} values ranging from 5.3 to 145.1 μM. Preliminary structure−activity analysis indicated that the terpenoid side chain and the phenolic hydroxy groups contributed greatly to the α-glucosidase inhibitory activity of **1**−**11**. In a cytotoxicity assay, compound **11** also presented weak cytotoxicity against two cell lines, A549 and HeLa, with IC_{50} values of 49.0 and 40.5 μM.



Hericium erinaceus, also known as lion's mane mushroom, is an important edible and medicinal mushroom. The fruiting bodies and mycelia of this mushroom have been used as an herbal medicine for the treatment of gastricism and hyperglycemia in China.[1] Aromatic compounds and diterpenoids with various interesting bioactivities have been isolated from *H. erinaceus*. Examples include hericenones C−E[2−4] and erinacines A−K,[5−10] possessing stimulatory activity for the biosynthesis of nerve growth factor, isohericenone and isohericerin with cytotoxicity,[11] (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxy-6-methoxy-2-phenethylisoindolin-1-one and (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxy-6-methoxyisoindolin-1-one with α-glucosidase inhibitory activity,[12] and methyl 4-hydroxy-3-(3-methylbutanoyl)benzoate, 2-chloro-1,3-dimethoxy-5-methylbenzene, methyl 4-chloro-3,5-dimethoxylbenzoate, and 4-chloro-3,5-dimethoxylbenzaldehyde, exhibiting a protective effect against endoplasmic reticulum stress-dependent cell death.[13]

It is well known that the different culture media can induce or inhibit different secondary metabolite biosynthetic gene clusters in the fermentation process of microorganisms.[14,15] In the past 10 years, mushrooms cultured in liquid have been studied for their pharmacological effects and bioactive components.[16,17] Solid-state fermentation (SSF) refers to the fermentation process using moist solid substrates in the absence or near absence of free-flowing water. Recently, we have separated a number of bioactive secondary metabolites from the solid culture of mushrooms, such as *Flammulina velutipes*,[18] *Pleurotus eryngii*,[19] *Pleurotus cornucopiae*,[20] and *Cyathus gansuensis*.[21]

During our expeditions to explore the fungal resources of the Tibet plateau region, a strain of *H. erinaceus* was isolated from its fruiting body collected in 2012. This fungus was fermented on rice and extracted with ethyl acetate to afford the organic solvent extract. High-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) analysis of this extract indicated the presence of many secondary metabolites. In order to obtain new bioactive secondary metabolites from this mushroom, a detailed chemical investigation was conducted, which led to the isolations of 10 new isoindolin-1-ones (**1**−**10**) together with the known compound (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxy-6-methoxy-2-phenethylisoindolin-1-one (**11**). In this work, we describe the isolation, structural elucidation, α-glucosidase inhibitory activity, and cytotoxicity of **1**−**11**.

RESULTS AND DISCUSSION

The ethyl acetate extract of the mushroom *H. erinaceus* fermented on rice was separated by silica gel and reversed-phase C_{18} column chromatography followed by semipreparative HPLC to give secondary metabolites **1**−**11** (Figure 1). The

Received: May 29, 2014

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

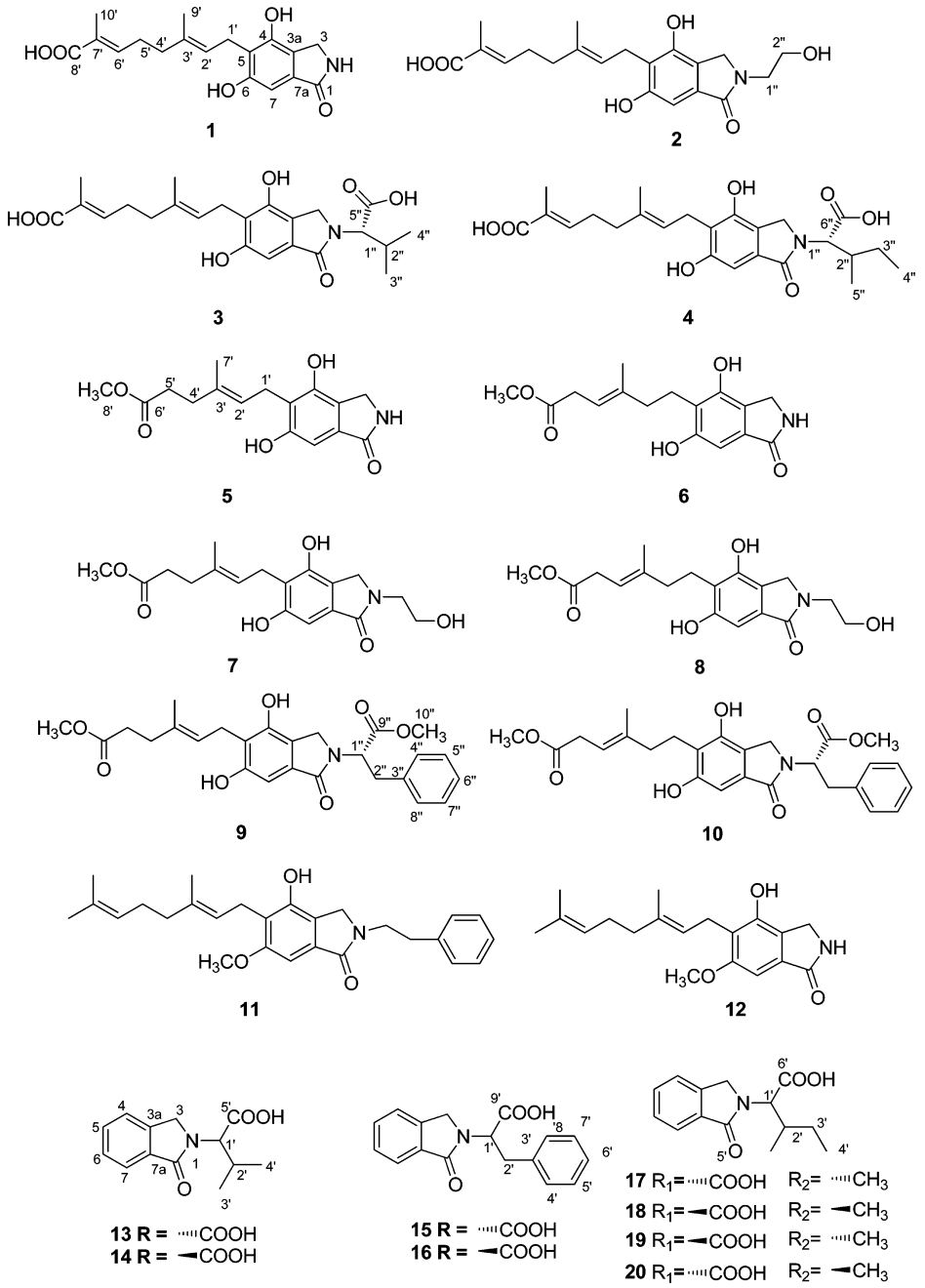


Figure 1. Structures of compounds **1**–**20**.

structure of the known compound (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4-hydroxy-6-methoxy-2-phenethylisoindolin-1-one (**11**) was determined by comparing the experimental NMR data obtained with literature values.[12]

Erinacerin C (**1**) was obtained as a colorless powder. It has a molecular formula of $C_{18}H_2NO_5$, as determined by the molecular ion peak at *m*/*z* 332.1496 $[M + H]^+$ obtained by HRTOFMS. The ^1H, ^{13}C, and HSQC NMR data of **1** showed resonances due to two olefinic methyl groups (δ_H 1.71 3H, s; 1.75, 3H, s; δ_C 12.2 and 16.0), four methylenes (δ_H 2.01, 2H, t, *J* = 7.4 Hz; 2.20, 2H, m; 3.29, 2H, d, *J* = 6.8 Hz; 4.13, 2H, s; δ_C 37.9, 26.8, 22.4, 43.0), one aromatic proton (δ_H 6.62, 1H, s; δ_C 100.3), two trisubstituted olefins [δ_H 5.22, 1H, t, *J* = 6.8 Hz; 6.60, 1H, t, *J* = 7.5 Hz; δ_C 123.0 (=CH), 133.2, 141.2 (=CH), 127.8], five tertiary aromatic carbons (δ_C 118.5, 121.0, 131.3, 150.4, and 156.2), and two carbonyl groups (δ_C 168.9 and 170.4). The ^1H and ^{13}C spectral data of **1** were similar to those of the known compound **12**,[12] except for the loss of one olefinic methyl group and the presence of an additional carbonyl moiety. All the above data indicate the presence of an isoindolinone skeleton in **1**. The HMBC correlations from H_2-3 to C-1, C-3a, C-4, and C-7a, from H-7 to C-1, C-3a, C-5, C-6, and C-7a, from H_2-1′ to C-2′, C-3′, C-4, C-5, and C-6, from H_2-5′ to C-3′, C-4′, C-6′, and C-7′, from H_3-9′ to C-2′, C-3′, and C-4′, and from H_3-10′ to C-6′, C-7′, and C-8′ supported the structure of **1** as shown in Figure 1. The chemical shifts for C-4 (δ_C 150.4), C-6 (δ_C 156.2), and C-8′ (δ_C 170.4), as well as the molecular formula of **1**, confirmed the substitution of a hydroxy group at C-4 and C-6 and the presence of a carboxylic acid group at C-8′. NOE correlations observed between H-2′

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

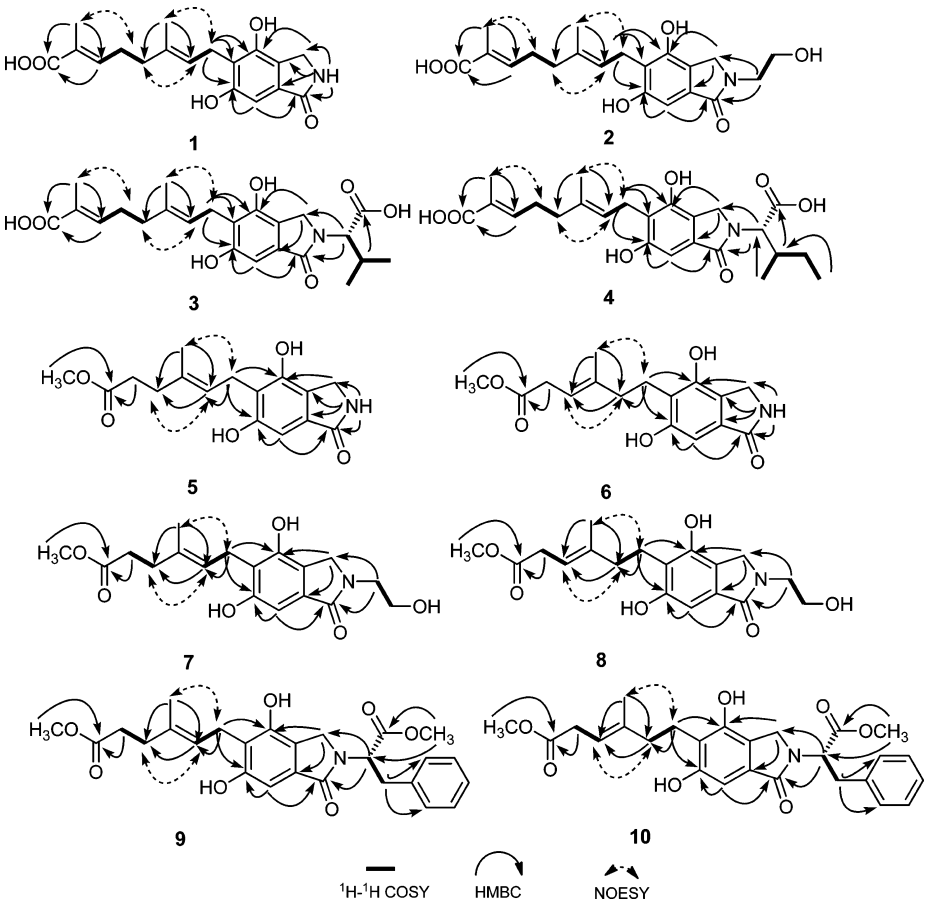


Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **1**−**10**.

and H_2-4′ and between H_2-5′ and H_3-10′ confirmed the *E* configuration for two double bonds. On the basis of above analysis, the structure of **1** was determined as (2*E*,6*E*)-8-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid.

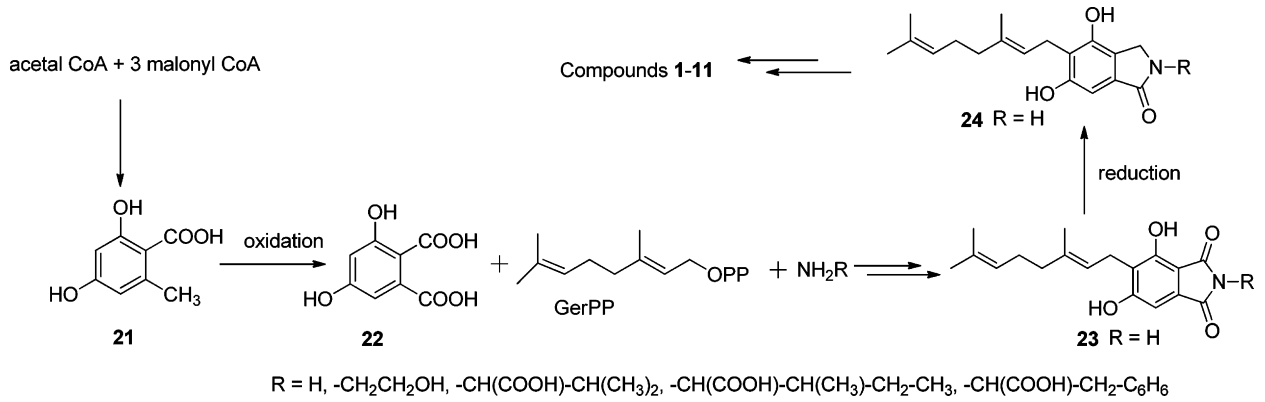
Compound **2** was isolated as a yellow powder. The molecular formula of **2** was determined to be $C_{20}H_{25}NO_6$ by the molecular ion peak at *m*/*z* 376.1757 $[M + H]^+$ in its HRTOFMS. The ^{1}H and ^{13}C NMR spectra of **2** were quite similar to those of **1**, except for the presence of two extra methylenes [δ_H 3.50 (2H, t, *J* = 5.3 Hz, H_2-1″); 3.57 (2H, t, *J* = 5.3 Hz, H_2-2″); δ_C 48.8 and 59.4] in **2**. The coupling constant between H_2-1″ and H_2-2″, as well as the HMBC correlations from H_2-1″ to C-1, C-3, and C-2″ and from H_2-2″ to C-1″, established the 2-hydroxyethyl moiety was linked to the nitrogen atom. NOE correlations of H_2-1′ with H_3-9′, H-2′ with H_2-4′, and H_2-5′ with H_3-10′ assigned the *E* configuration for two double bonds. Thus, the structure of **2** was determined as (2*E*,6*E*)-8-(4,6-dihydroxy-2-(2-hydroxyethyl)-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid. It was designated as erinacerin D.

A molecular formula of $C_{23}H_{29}NO_7$ was assigned for **3** on the basis of its HRTOFMS and NMR data analysis. The ^{1}H and ^{13}C NMR NMR and IR data for **3** showed that it was a structural analogue of **1**, sharing the same substructure of (2*E*,6*E*)-8-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid. Compound **3** differs from **1** in the nitrogen-linked moiety. The ^{1}H−^{1}H COSY correlations between H-1″ (δ_H 4.45, d, *J* = 8.1 Hz) and H_2-2″ (δ_H 2.22, 2H, m), between H-2″ and H_3-3″ (δ_H 0.82, 3H, d, *J* = 6.8 Hz), and between H-2″ and H_3-4″ (δ_H 0.99, 3H, d, *J* = 6.8 Hz) together with the HMBC correlations from H_2-1″ to C-1 (δ_C 168.8), C-3 (δ_C 45.3), C-2″ (δ_C 28.2), C-3″ (δ_C 19.5), C-4″ (δ_C 19.0), and C-5″ (δ_C 172.0), from H-2″ to C-4″ and C-5″, and from H_3-3″(4″) to C-1″ (δ_C 59.7) and C-2″ confirmed the connection of a 3-methylbutyric acid moiety at the nitrogen atom (Figure 2). Assignments of the ^{1}H and ^{13}C signals in **3** were achieved by detailed analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Figure 2). In the ROESY spectrum of **3**, NOE correlations of H_2-1′ with H_3-9′, H-2′ with H_2-4′, and H_2-5′ with H_3-10′ were observed, which determined the *E* configuration for two double bonds. On the basis of the above analysis, the structure of **3** was determined as (2*E*,6*E*)-8-(2-(1-carboxy-2-methylpropyl)-4,6-dihydroxy-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid. It was named erinacerin E.

Erinacerin F (**4**) was obtained as a yellow oil. Its molecular formula of $C_{24}H_{31}NO_7$ was assigned by the molecular ion peak for $[M + H]^+$ at *m*/*z* 446.2170 in its HRTOFMS. Comparison of the NMR data between **3** and **4** suggested that they had the same substructure of (2*E*,6*E*)-8-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid, with the main difference at the nitrogen-linked moiety. Specifically, compound **4** exhibited the presence of an additional methylene (δ_H 1.28, 2H, m; δ_C 34.2). The HMBC correlations from H-1″ (δ_H 4.53, 1H, d, *J* = 9.7 Hz) to C-1 (δ_C 168.8), C-3 (δ_C 45.2), C-2″ (δ_C 24.9), C-3″ (δ_C 34.2), C-5″ (δ_C 16.0), and C-6″ (δ_C 172.1), from H-2″ (δ_H 2.24, 2H, m) to C-1″ (δ_C 58.2), C-3″, C-4″ (δ_C 10.7), C-5″, and C-6″, from H_2-3″ (δ_H 1.28, 2H, m) to C-1″, C-2″, C-4″, and C-5″, from H_3-4″ (δ_H 0.84, 3H, s) to C-2″ and C-3″, and from H_3-5″ (δ_H 0.96, 3H, d, *J* = 6.7 Hz) to C-1″, C-2″,

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 1. Hypothetical Biogenetic Pathway of 1−11.



and C-3″ indicated the linkage of a 1-carboxy-2-methylbutyl moiety at the nitrogen atom. NOE correlations of H_2-1′ with H_3-9′, H-2′ with H_2-4′, and H_2-5′ with H_3-10′ confirmed the *E* configuration for two double bonds. Therefore, the structure of **4** was concluded as (2*E*,6*E*)-8-(2-(1-carboxy-2-methylbutyl)-4,6-dihydroxy-1-oxoisoindolin-5-yl)-2,6-dimethylocta-2,6-dienoic acid.

The same molecular formula of $C_{16}H_{19}NO_5$ was determined for erinacerin G (**5**) and erinacerin H (**6**) by HRTOFMS data analysis. The ^{1}H and ^{13}C NMR spectra of **5** and **6** indicated the presence of a 5-substituted-4,6-dihydroxyisoindolin-1-one moiety in their structures. A comparison of NMR data of **5** and **6** with those obtained for **1** revealed the presence of an extra methoxyl group (**5**: δ_H 3.52, 3H, s; δ_C 51.6; **6**: δ_H 3.59, 3H, s; δ_C 51.9) and the loss of a trisubstituted double bond and a methyl group, which indicated the structural variation in the side chain located at C-5 in the benzene ring for these two compounds. The important HMBC correlations of H_2-1′ with C-4, C-5, C-6, C-2′, and C-3′; H-2′ with C-5, C-1′, C-3′, C-4′, and C-7′; H_2-4′ with C-2′, C-3′, C-5′, C-6′, and C-7′; H_2-5′ with C-3′, C-4′, and C-6′; and H_3-8′ with C-6′ (Figure 2), together with the NOE correlations of H-2′ with H_2-4′ and H_2-1′ with H_3-7′ (Figure 2), assigned the structure of **5** as (*E*)-methyl 6-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-4-methylhex-4-enoate. Similarly, a detailed interpretation of the HMBC and ROESY spectra of **6** determined its structure as (*E*)-methyl 6-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-4-methylhex-3-enoate (Figure 2). Compounds **5** and **6** are a pair of double-bond positional isomers.

Compounds **7** and **8** were assigned the same molecular formula of $C_{18}H_{23}NO_6$ on the basis of their HRTOFMS and NMR data analysis. The ^{1}H and ^{13}C NMR spectra of **7** were very similar to those of compound **5**, with the exception of the presence of two additional methylenes (δ_H 3.69, 2H, t, J = 5.4 Hz, H_2-1″; 3.79, 2H, t, J = 5.4 Hz, H_2-2″; δ_C 46.3 and 61.3). The ^{1}H−^{1}H COSY between H_2-1″ and H_2-2″ in combination with the HMBC correlations from H_2-1″ to C-1, C-3, and C-2″ and from H_2-2″ to C-1″ confirmed the linkage of the 2-hydroxyethyl moiety at the nitrogen atom. The NOE correlations of H-2′ with H_2-4′ and of H-1′ with H_3-7′ determined the *E* configuration for the double bond between C-2′ and C-3′. Accordingly, the structure of **7** was elucidated as (*E*)-methyl 6-(4,6-dihydroxy-2-(2-hydroxyethyl)-1-oxoisoindolin-5-yl)-4-methylhex-4-enoate. Compound **8** has similar ^{1}H and ^{13}C NMR spectra to those of compound **6**, except for two extra methylenes. The interpretation of HMBC and ROESY spectra of **8** assigned its structure as (*E*)-methyl 6-(4,6-dihydroxy-2-(2-hydroxyethyl)-1-oxoisoindolin-5-yl)-4-methylhex-3-enoate (Figure 2). Compounds **7** and **8** are a pair of double-bond positional isomers. They were designated as erinacerins I and J, respectively.

Erinacerins K (**9**) and L (**10**) were isolated as yellow oils. They possessed the same molecular formula of $C_{26}H_{29}NO_7$, as determined by the HRTOFMS and NMR data analysis. In the ^{1}H and ^{13}C NMR spectra of **9**, signals corresponding to the substructure of (*E*)-methyl 6-(4,6-dihydroxy-1-oxoisoindolin-5-yl)-4-methylhex-4-enoate moiety, a monosubstituted aromatic ring [δ_H 7.23 (4H, overlapped); 7.16 (1H, m); δ_C 126.6, 128.4 (two carbons), 128.6 (two carbons), 137.1], a methoxyl group (δ_H 3.66, 3H, s; δ_C 52.3), a methine group (δ_H 5.15, 1H, m; δ_C 54.7), a methylene [δ_H 3.14, 1H, dd, J = 11.0, 14.5 Hz, 3.37 (overlapped); δ_C 34.7], and an ester ketone (δ_C 170.9) were present. Furthermore, the ^{1}H−^{1}H COSY and HMBC data analysis supported the presence of a methyl 3-phenylpropanoate moiety in **9**. The HMBC correlations from H-1″ to C-1 and C-3 confirmed the connectivity of the methyl 3-phenylpropanoate moiety with the nitrogen atom in **9** (Figure 2). NOE correlations of H-2′ with H_2-4′ and of H-1′ with H_3-7′ assigned the *E* configuration for the double bond. Thus, the structure of **9** was established as described in Figure 1. In a similar way, compound **10** was determined as shown by analysis of its 2D NMR data (Figure 2).

To determine the absolute configuration in compounds **3**, **4**, **9**, and **10**, phthalimidines **13**−**20** were synthesized by the reaction of *o*-phthalaldehyde with the *α*-amino acid (L-valine, D-valine, L-phenylalanine, D-phenylalanine, D-isoleucine, L-isoleucine, D-alloisoleucine, and L-alloleucine).[22] The absolute configurations at C-1″ in compounds **3**, **9**, and **10** were determined by comparsion of their specific optical rotations with those of the synthetic phthalimidines **13**−**16**. Compounds **3**, **9**, and **10** showed negative specific optical rotations, indicating the *S* configuration at C-1″ in compounds **3**, **9**, and **10**. The chemical shifts of the amino acid moiety in **4** were consistent with those of **17** and **18**. The stereochemistry in **4** was established as 1″*S* and 2″*S* by comparing its specific optical rotation (−20.1) with that of **17** (−40.3) and **18** (+58.09).

The possible biogenetic pathway of **1**−**11** is proposed as shown in Scheme 1. The key presumed intermediate orsellicic acid (**21**) is biosynthesized from the acetyl malonyl pathway. Taking the biosynthesis of compound **1** as an example, compound **21** could be oxidized into 3,5-dihydroxyphthalic acid (**22**), which subsequently reacts with geranyl pyrophosphate (GerPP) and ammonia to give (*E*)-5-(3,7-dimethylocta-2,6-dien-1-yl)-4,6-dihydroxyisoindoline-1,3-dione (**23**). Com-

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1−4

	1 (DMSO-d_6)		2 (DMSO-d_6)		3 (DMSO-d_6)		4 (DMSO-d_6)	
no.	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)
1	168.9		167.8		168.3		168.3	
2		8.27 s						
3	43.0	4.13 s	44.7	4.32 s	45.3	4.42 d (17.0); 4.24 d (17.0)	45.2	4.45 d (17.0); 4.22 d (17.0)
3a	131.3		131.3		130.0		130.0	
4	150.4		150.1		150.3		150.2	
5	121.0		119.0		119.0		119.0	
6	156.2		156.1		156.4		156.4	
7	100.3	6.62 s	100.3	6.63 s	100.5	6.65 s	100.5	6.66 s
7a	118.5		118.5		119.1		119.1	
1′	22.4	3.29 d (6.8)	22.4	3.29 d (6.9)	22.5	3.30 d (7.1)	22.4	3.30 d (7.1)
2′	123.0	5.22 t (6.8)	123.0	5.21 t (6.9)	122.8	5.21 t (7.1)	122.8	5.22 t (7.1)
3′	133.2		133.2		133.3		133.3	
4′	37.9	2.01 t (7.4)	37.9	2.01 t (7.3)	37.9	2.00 t (7.4)	37.9	2.01 t (7.4)
5′	26.8	2.20 m	26.9	2.20 m	26.9	2.19 m	26.9	2.20 m
6′	141.2	6.60 t (7.5)	141.2	6.60 t (7.4)	141.3	6.60 t (7.3)	141.3	6.61 t (7.3)
7′	127.8		127.8		127.7		127.7	
8′	170.4		169.0		168.8		168.8	
9′	16.0	1.75 s	16.0	1.75 s	16.0	1.74 s	15.7	1.75 s
10′	12.2	1.71 s	12.2	1.71 s	12.2	1.70 s	12.2	1.71 s
1″			48.8	3.50 t (5.3)	59.7	4.45 d (8.1)	58.2	4.53 d (9.7)
2″			59.4	3.57 t (5.3)	28.2	2.22 m	24.9	2.24 m
3″					19.5	0.82 d (6.7)	34.2	1.28 m
4″					19.0	0.99 d (6.7)	10.7	0.84 t (7.0)
5″					172.0		16.0	0.96 d (6.7)
6″							172.1	
4-OH		9.21 s		9.26 s		9.31 s		9.31 s
6-OH		9.48 s		9.50 s		9.57 s		9.58 s

pound **23** is transformed into isoindolin derivative **24** by reduction. Finally, compound **1** was synthesized from **24** by oxidation. Compounds **2**−**11** may be biosynthesized from **21** by the same route as that proposed for **1**.

Isoindolin-1-ones incorporating a terpenoid moiety are a group of fungal metabolites isolated from the fungus of *H. erinaceus*[12] and *Stachybotrys* species.[23−26] They have been reported to possess various interesting bioactivities, such as inhibition of thromboxane A_2-induced vasoconstriction,[23] inhibitory activity against α-glucosidase,[12] inhibition of tyrosine kinase,[24] inhibition of HIV-1 protease,[25] and antichloesterolemic, anti-inflammatory, and cytotoxic activities.[26]

The crude extracts of *H. erinaceus* have been reported to possess antihyperglycemic effects.[27] In this report, we utilized an *in vitro* α-glucosidase inhibition assay to evaluate the antihyperglycemic effect of compounds **1**−**11**. The known compound **11** showed the strongest inhibitory activity with an IC_{50} of 5.3 μM, which was consistent with the early report.[12] Compound **1** showed weak inhibition against α-glucosidase with an IC_{50} value larger than 200 μM. New isoindolin-1-ones **2**−**10** exhibited α-glucosidase inhibitory activity with IC_{50} values of 24.2, 12.5, 39.6, 145.1, 10.3, 97.8, 18.6, 15.5, and 19.8 μM, respectively (Table 4). On the basis of the above activity data, a preliminary structure−activity relationship was deduced. The substitution on the nitrogen atom can increase the inhibitory activity, as indicated by comparing the inhibitory activity of **1** with that of **2**−**4**. Compounds **3**, **4**, **9**, and **10** showed stronger inhibitory activity than compounds **13**−**17**, which indicated the significance of the terpenoid side chain and the phenolic hydroxy groups for the α-glucosidase inhibitory activity. Compounds **5** and **6** showed stronger inhibitory activities than **1**, suggesting that the length and stereochemistry of the terpenoid side chain on the benzene ring also influence the activity.

To evaluate the anticancer activity for all isolates, we tested the cytotoxicity of compounds **1**−**11** and **13**−**17** toward two cancer cell lines, human lung adenocarcinoma cell line A549 and cervical cancer cell line HeLa. As seen in Table 4, the known compound **11** presented much stronger activity against the growth of A549 (IC_{50} = 49.0 μM) and HeLa (IC_{50} = 40.5 μM) than the other isolates. Compounds **8**, **9**, **13**, and **14** showed weak cytotoxic activity against A549 with IC_{50} values of 96.1, 89.7, 89.7, and 41.5 μM, respectively.

In summary, 10 new isoindolin-1-ones (**1**−**10**) with α-glucosidase inhibitory activity were obtained from the solid culture of the medicinal mushroom *H. erinaceus*, which supported the medicinal value of this mushroom and expanded the chemistry of isoindolin-1-ones. The known compound **11** that was previously isolated from the fruiting bodies of *H. erinaceus* was found to possess strong α-glucosidase inhibitory activity and weak cytotoxicity. On the basis of the current work and our previous publications,[18−21] the solid-state fermentation technique is demonstrated as a useful method to stimulate the biosynthesis of secondary metabolites in edible and medicinal mushrooms.

EXPERIMENTAL SECTION

General Experimental Procedures. Solvents used for extraction and chromatographic separation were analytical grade. TLC was carried out on silica gel $HSGF_{254}$, and the spots were visualized by spraying with 10% H_2SO_4 and heating. Silica gel (Qingdao Haiyang Chemical Co., Ltd., People's Republic of China) and Sephadex LH-20

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

(Amersham Biosciences) were used for column chromatography. HPLC separation was performed on an Agilent 1200 HPLC system using an ODS column (C_{18}, 250 × 9.4 mm, YMC Pak, 5 μm; detector: UV) with a flow rate of 2.0 mL/min. UV and IR spectral data were acquired using a ThermoGenesys-10S UV−vis and Nicolet IS5FT-IR spectrophotometer, respectively. Specific rotations were recorded on a PerkinElmer 241 polarimeter. NMR spectral data were obtained with a Bruker Avance-500 spectrometer (DMSO-d_6, δ_H 2.50/δ_C 40.0; methanol-d_4, δ_H 3.30/δ_C 49.9). The HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. HR-TOF-MS data were measured using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument.

Fungal Material. The strain used in this work was isolated from the fruiting body of *H. erinaceus* and identified by one of the authors (Y.P.). The fungus was identified on the basis of the DNA sequences of the ITS1-5.8S-ITS2 regions of their rRNA gene. The ITS gene sequence obtained in this study (accession number: KJ627741) showed 99% homology with that of the fungus *H. erinaceus* in GenBank with the accession number GU566758. *H. erinaceus* was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were inoculated in 500 mL Erlenmeyer flasks containing 120 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract; the final pH of the media was adjusted to 6.5 before sterilization) and incubated at 25 °C on a rotary shaker at 170 rpm for 1 week. The scale-up fermentation was carried out in 30 500 mL Fernbach culture flasks each containing 80 g of rice and 120 mL of distilled water. Each flask was inoculated with 5.0 mL of the culture medium and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented rice substrate was extracted repeatedly with EtOAc (3 × 4L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (19.6 g). The residue (18.0 g) was subjected to silica gel column chromatography (CC) using hexane−ethyl acetate in a gradient elution (v/v, 100:0, 100:2, 100:5, 100:8, 100:10, 100:15, 100:20, 100:40, 100:50), followed by dichloromethane−methanol elution (v/v, 100:1, 100:2, 100:3, 100:5, 100:10, 100:15, 100:20, 0:100) to give 20 fractions (HE-1−HE-20).

Fraction 14 (1.45 g), eluted with dichloromethane−methanol (v/v, 100:2), was further separated on Sephadex LH-20 CC eluted with 50% methanol in water to give 20 subfractions (HE-14-1−HE-14-20). Fraction HE-14-12 (89 mg) was further separated by Sephadex LH-20 CC eluted with 50% methanol in water to afford compounds **3** (7 mg) and **4** (5.4 mg). Compound **11** (12.1 mg, t_R 40 min) was obtained from fraction HE-14-16 (45.2 mg) by RP-HPLC using 65% methanol in water. Fraction HE-19 (3.45g), eluted with dichloromethane−methanol (v/v, 100:5), was first separated by ODS CC using a gradient of methanol−water (20−100%) to afford 30 subfractions (HE-19-1−HE-19-30). Compounds **1** (82 mg, t_R 35.3 min) and **2** (5.3 mg, t_R 34.2 min) were obtained from fraction HE-19-15 (213.5 mg) by RP-HPLC using 30% acetonitrile in water. Fraction HE-19-12 (51.2 mg) was subjected to Sephadex LH-20 CC eluted with 50% methanol in water to afford four subfractions (HE-19-12-1−HE-19-12-4). Compounds **5** (3.6 mg, t_R 36.3 min) and **6** (1.2 mg, t_R 35.5 min) were obtained from subfraction HE-19-12-4 (12.5 mg) by RP-HPLC using 22% acetonitrile in water. Compounds **7** (5.1 mg, t_R 26.5 min) and **8** (2.8 mg, t_R 25.7 min) were purified from HE-19-12-3 (13.1 mg) by RP-HPLC using 22% acetonitrile in water. Subfraction HE-19-16 (21.5 mg) was separated by RP-HPLC using 40% acetonitrile in water to afford compounds **9** (5.2 mg, t_R 40.6 min) and **10** (1.8 mg, t_R 42.1 min). The physical properties and spectroscopic data of the new compounds are as follows.

Erinacerin C (1): colorless powder; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3272, 2924, 2855, 1685, 1604, 1460, 1381, 1271, 1164, 1060, 825, 738, 600 cm^{-1}; for ^{1}H and ^{13}C NMR data measured in DMSO-d_6 see Table 1; for ^{1}H and ^{13}C NMR data measured in CD_3OD see Table S1 in the Supporting Information; positive HRTOFMS m/z [M + H]$^+$ 332.1498 (calcd for $C_{18}H_{22}NO_5$, 332.1496).

Erinacerin D (2): yellow powder; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3306, 2928, 1673, 1464, 1349, 1204, 1142, 1061, 833, 800, 735, 612 cm^{-1}; for ^{1}H and ^{13}C NMR data measured in DMSO-d_6 see Table 1; for ^{1}H and ^{13}C NMR data measured in CD_3OD see Table S1 in the Supporting Information; positive HRTOFMS m/z [M + H]$^+$376.1757 (calcd for $C_{20}H_{26}NO_6$, 376.1755).

Erinacerin E (3): yellow oil; $[\alpha]_D^{25}$ −19.5 (c 1.0, methanol); UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3222, 2965, 2925, 1673, 1462, 1393, 1358, 1203, 1024 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 1; positive HRTOFMS m/z [M + H]$^+$ 432.2021 (calcd for $C_{23}H_{30}NO_7$, 432.2017).

Erinacerin F (4): yellow oil; $[\alpha]_D^{25}$ −20.1 (c 0.6, methanol); UV(methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3210, 2938, 2920, 1675, 1463, 1350, 1222, 1020, 853, 750, 620 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 1; positive HRTOFMS m/z [M + H]$^+$ 446.2170 (calcd for $C_{24}H_{34}NO_7$, 446.2173).

Erinacerin G (5): colorless oil; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3273, 2948, 2842, 1680, 1470, 1328, 1240, 1020, 856, 789, 610 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; positive HRTOFMS m/z [M + H]$^+$ 306.1338 (calcd for $C_{16}H_{20}NO_5$, 306.1336).

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 5−7

	5 (DMSO-d_6)		**6** (DMSO-d_6)		**7** (CD_3OD)	
position	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)
1	170.9		170.9		171.4	
3	43.4	4.14 s	43.4	4.13 s	50.6	4.43 s
3a	131.8		131.8		132.0	
4	150.8		151.0		151.6	
5	118.8		119.6		121.3	
6	156.6		156.7		158.3	
7	100.7	6.63 s	100.7	6.63 s	101.8	6.74 s
7a	121.4		121.4		120.8	
1′	22.9	3.28 d (7.1)	22.5	2.69 t (7.1)	23.6	3.40 d (7.1)
2′	123.6	5.20 t (7.1)	38.6	2.10 t (7.1)	124.6	5.28 t (7.1)
3′	132.9		139.2		134.2	
4′	34.7	2.17 t (7.6)	116.0	5.26 t (7.0)	36.0	2.26 t (7.5)
5′	32.8	2.35 t (7.6)	33.4	3.06 d (7.0)	33.9	2.39 t (7.5)
6′	173.5		172.5		175.1	
7′	16.3	1.73 s	16.8	1.67 s	16.1	1.80 s
8′	51.6	3.52 s	51.9	3.59 s	51.9	3.56 s
1″					46.3	3.69 t (5.4)
2″					61.3	3.79 t (5.4)
4-OH		9.25 s		9.18 s		
6-OH		9.51 s		9.47 s		
NH		8.28 s		8.27 s		

Erinacerin H (6): colorless oil; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3270, 2948, 2832, 1667, 1470, 1340, 1246, 1018, 1007, 880, 790, 625 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; positive HRTOFMS m/z [M + H]$^+$ 306.1338 (calcd for $C_{16}H_{20}NO_5$, 306.1336).

Erinacerin I (7): colorless powder; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3270, 2940, 2854, 1711, 1658, 1605, 1465, 1348, 1304, 1205, 1164, 1061, 860, 788, 620 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 2; positive HRTOFMS m/z [M + H]$^+$ 350.1593 (calcd for $C_{18}H_{24}NO_6$, 350.1598).

Erinacerin J (8): yellow oil; UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3267, 2952, 2860, 1659, 1459, 1364, 1300, 1240, 870, 756 cm^{-1}; for ^{1}H and ^{13}C NMR data see

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{1}H and ^{13}C NMR Data of Compounds 8−10

	8 (DMSO-d_6)		9 (DMSO-d_6)		10 (DMSO-d_6)	
no.	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)
1	167.8		168.1		168.6	
3	48.8	4.33 s	45.5	4.14 d (16.6); 4.23 d (16.6)	46.0	4.16 d (16.6); 4.24 d (16.6)
3a	131.4		130.0		130.5	
4	150.2		150.2		150.8	
5	119.0		119.0		120.4	
6	156.3		156.4		156.9	
7	100.3	6.64 s	100.5	6.58 s	100.9	6.60 s
7a	119.1		119.2		119.4	
1′	22.1	2.69 t (7 0.0)	22.4	3.25 d (8.1)	22.5	2.67 t (7,0)
2′	38.2	2.10 t (7.0)	122.9	5.15[b]	38.5	2.08 t (7.0)
3′	139.0		132.7		139.1	
4′	115.5	5.26 t (7.0)	32.3	2.33 t (7.6)	116.0	5.22 t (6.5)
5′	33.0	3.05 d (7.0)	34.3	2.15 t (7.6)	33.4	3.05 d (6.5)
6′	172.1		173.0		172.5	
7′	16.3	1.66 s	15.9	1.71 s	16.8	1.66 s
8′	51.5	3.58[a]	51.1	3.49 s	51.9	3.58 s
1″	44.7	3.50 t (5.5)	54.7	5.15[b]	55.2	5.16 dd (6.0, 11.0)
2″	59.5	3.58[a]	34.7	3.14 dd (11.2, 14.5); 3.37[c]	35.2	3.16 dd (11.0, 14.5) 3.37[c]
3″			137.1		137.5	
4″, 8″			128.6	7.23[d]	128.9	7.24[e]
5″, 7″			128.4	7.23[d]	129.0	7.24[e]
6″			126.6	7.16 m	127.1	7.17 m
9″			170.9		171.4	
10″			52.3	3.66 s	52.8	3.66 s
4-OH		9.23 s		9.28 s		9.26 s
6-OH		9.49 s		9.57 s		9.58 s

[a,b,d,e]Signals overlapped with each other. [c]Signals overlapped with residual H_2O signal.

Table 3; positive HRTOFMS m/z $[M + H]^+$ 350.1595 (calcd for $C_{18}H_{24}NO_6$, 350.1598).

Erinacerin K (9): yellow oil; $[\alpha]_D^{25}$ −65.4 (c 1.2, methanol); UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3270, 2952, 2925, 1737, 1667, 1459, 1355, 1295, 1210, 1153, 875, 740, 615 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 3; positive HRTOFMS m/z $[M + H]^+$ 468.2017 (calcd for $C_{26}H_{30}NO_7$, 468.2017).

Erinacerin L (10): yellow oil; $[\alpha]_D^{25}$ −65.3 (c 1.2, methanol); UV (methanol) λ_{max} nm (log ε) 213 (4.63), 262 (4.29), 305 (2.12); IR (neat) ν_{max} 3275, 2923, 2863, 1659, 1464, 1325, 1298, 1220, 1154, 880, 728, 628 cm^{-1}; for ^{1}H and ^{13}C NMR data see Table 3; positive HRTOFMS m/z $[M + H]^+$ 468.2019 (calcd for $C_{26}H_{30}NO_7$, 468.2017).

Table 4. α-Glucosidase Inhibtory and Cytotoxic Activity of Compounds 1−11 and 13−17

		cytotoxic activity	
compound	α-glucosidase inhibition (IC_{50}, μM)	A549 (IC_{50}, μM)	HeLa (IC_{50}, μM)
1	>200	>100	>100
2	24.2	>100	>100
3	12.8	>100	>100
4	39.6	>100	>100
5	145.1	>100	>100
6	10.3	>100	>100
7	97.8	>100	>100
8	18.6	96.1	>100
9	15.5	89.7	>100
10	19.8	>100	>100
11	5.3	49.0	40.5
13	>200	89.7	>100
14	>200	41.5	>100
15	>200	>100	>100
16	>200	>100	>100
17	>200	>100	>100
positive control	acarbose 382.7	cisplatin 12.6	cisplatin 14.4

Synthesis of Phthalimidines (13−20).[22] L-Valine (2 mmol) was added into a solution of phathalic dicarboxaldehyde (2 mmol) in 30 mL of acetonitrile. The mixture was heated under reluxing for 16 h; then the reaction mixture was filtered while hot. The solvent was allowed to cool to yield the desired phathalimidine **13** as crystalline solids (0.42 g). Using the same method, compounds **14** (0.40 g), **15** (0.52 g), **16** (0.48 g), **17** (0.46 g), **18** (0.23 g), **19** (0.15 g), and **20** (0.23 g) were synthesized from L-valine, D-valine, L-phenylalanine, D-phenylalanine, D-isoleucine, L-isoleucine, D-alloisoleucine, and L-alloleucine, respectively.

Compound 13: colorless crystalline; $[\alpha]_D^{25}$ −41.8 (c 1.0, methanol); IR (neat) ν_{max} 1654,1434, 1320, 1229, 1124, 860, 732, 624 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) 13.01 (1H, s, H-5″), 7.71 (1H, d, J = 7.4 Hz, H-7), 7.63 (1H, d, J = 7.4 Hz, H-4), 7.62 (1H, m, H-5), 7.50 (1H, m, H-6), 4.63 (1H, d, J = 17.5 Hz, H-3), 4.53 (1H, d, J = 17.5 Hz, H-3), 4.51 (1H, d, J = 6.3 Hz, H-1″), 2.28 (1H, m, H-2″), 1.01 (3H, d, J = 6.7 Hz, H-3″), 0.83 (3H, d, J = 6.7 Hz, H-4″); ^{13}C NMR (125 MHz, DMSO-d_6) 172.3 (C-5″), 168.4 (C-1), 142.6 (C-3a), 132.1 (C-5), 131.8 (C-7a), 128.4 (C-6), 124.1 (C-4), 123.5 (C-7), 60.2 (C-1″), 47.7 (C-3), 28.6 (C-2″), 19.9 (C-3″), and 19.6 (C-4″); positive HRTOFMS m/z $[M + H]^+$ 234.1128 (calcd for $C_{13}H_{15}NO_3$, 234.1125).

Compound 14: colorless crystalline; $[\alpha]_D^{25}$ +40.9 (c1.0, methanol); the IR and ^{1}H and ^{13}C NMR data of **14** are identical with those of **13**; positive HRTOFMS m/z $[M + H]^+$ 234.1130 (calcd for $C_{13}H_{15}NO_3$, 234.1125).

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

Compound 15: yellow crystalline; $[\alpha]_D^{25}$ −75.1 (c 1.0, methanol); IR (neat) ν_{max} 1664, 1460, 1324, 1220, 1124, 864, 632 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 13.16 (1H, s, H-9″), 7.62 (1H, d, J = 7.1 Hz, H-7), 7.58 (1H, m, H-4), 7.56 (1H, m, H-5), 7.45 (1H, t, J = 7.1 Hz, H-6), 7.23 (4H, m, H-4″, H-5″, H-7″, H-8″), 7.14 (1H, t, J = 7.1 Hz), 5.14 (1H, dd, J = 11.4, 4.7 Hz, H-1″), 4.44 (2H, s, H-3), 3.40 (1H, dd, J = 14.7, 4.7 Hz, H-2″), 3.21 (1H, m, H-2″); ^{13}C NMR (125 MHz, DMSO-d_6) 172.0 (C-9″), 167.8 (C-1), 137.5 (C-3″), 131.7 (C-5), 131.6 (C-7a), 128.5 (C-4″, C-8″), 128.4 (C-5″, C-7″), 127.9 (C-6), 126.5 (C-6″), 123.5 (C-7), 122.9 (C-4), 54.8 (C-1″), 47.4 (C-3), 34.7 (C-2″); positive HRTOFMS m/z $[M + H]^+$ 282.1122 (calcd for $C_{17}H_{15}NO_3$, 282.1125).

Compound 16: yellow crystalline; $[\alpha]_D^{25}$ +91.0 (c 1.0, methanol); the IR and ^1H and ^{13}C NMR data of **16** are identical with those of **15**; positive HRTOFMS m/z $[M + H]^+$ 282.1130 (calcd for $C_{17}H_{15}NO_3$, 282.1125).

Compound 17: colorless crystalline; $[\alpha]_D^{25}$ −40.3 (c 1.0, methanol); IR (neat) ν_{max} 1665,1464, 1324, 1256, 1210, 728, 628 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 13.01 (1H, s, H-5″), 7.71 (1H, d, J = 7.4 Hz, H-7), 7.63 (1H, d, J = 7.4 Hz, H-4), 7.62 (1H, m, H-5), 7.50 (1H, m, H-6), 4.65 (1H, d, J = 17.5 Hz, H-3), 4.48 (1H, d, J = 17.5 Hz, H-3), 4.61 (1H, d, J = 9.7 Hz, H-1″), 2.11 (1H, m, H-3″), 1.33 (1H, m, H-2″), 1.09 (1H, m, H-2″), 0.98 (3H, d, J = 6.7 Hz, H-5″), 0.83 (3H, t, J = 7.4 Hz, H-4″); ^{13}C NMR (125 MHz, DMSO-d_6) 172.0 (C-5″), 167.9 (C-1), 142.5 (C-3a), 131.7 (C-5), 131.3 (C-7a), 128.0 (C-6), 123.6 (C-4), 123.0 (C-7), 58.3 (C-1″), 47.2 (C-3), 34.2 (C-3″), 25.0 (C-2″), 15.7 (C-5″), 10.6 (C-4″); positive HRTOFMS m/z $[M + H]^+$ 248.1280 (calcd for $C_{14}H_{17}NO_3$, 248.1281).

Compound 18: colorless crystalline; $[\alpha]_D^{25}$ +58.09 (c 1.0, methanol); the IR and ^1H and^{13}C NMR data of **18** are identical with those of **17**; positive HRTOFMS m/z $[M + H]^+$ 248.1282 (calcd for $C_{14}H_{17}NO_3$, 248.1281).

Compound 19: colorless crystalline; $[\alpha]_D^{25}$ +17.8 (c 1.0, methanol); IR (neat) ν_{max} 1665, 1423, 1310, 1256, 1220, 725, 625 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) 13.01 (1H, s, H-5″), 7.71 (1H, d, J = 7.4 Hz, H-7), 7.63 (1H, d, J = 7.4 Hz, H-4), 7.62 (1H, m, H-5), 7.50 (1H, m, H-6), 4.71 (1H, d, J = 9.7 Hz, H-1″), 4.64 (1H, d, J = 17.5 Hz, H-3), 4.51 (1H, d, J = 17.5 Hz, H-3), 2.11 (1H, m, H-3″), 1.33 (1H, m,, H-2″), 1.09 (1H, m, H-2″), 0.92 (3H, t, J = 7.4 Hz, H-4″), 0.86 (3H, d, J=6.7 Hz, H-5″); ^{13}C NMR (125 MHz, DMSO-d_6) 172.1 (C-5″), 168.1 (C-1), 142.2 (C-3a), 131.7 (C-5), 131.2 (C-7a), 127.9 (C-6), 123.6 (C-4), 123.0 (C-7), 57.8 (C-1″), 47.7 (C-3), 34.6 (C-3″), 25.8 (C-2″), 15.6 (C-4″), 11.1 (C-5″); positive HRTOFMS m/z $[M + H]^+$ 248.1285 (calcd for $C_{14}H_{17}NO_3$, 248.1281).

Compound 20: colorless crystalline; $[\alpha]_D^{25}$ −65.89 (c 1.0, methanol); the IR and ^1H and^{13}C NMR data of **20** are identical with those of **19**; positive HRTOFMS m/z $[M + H]^+$ 248.1282 (calcd for $C_{14}H_{17}NO_3$, 248.1281).

***α*-Glucosidase Inhibitory Assay.** As described in our early work,[28] the bioassay was conducted using a 96-well plate, and the absorbance was determined at 405 nm using a Spectra Max 190 microplate reader (Molecular Devices Inc.). The control was prepared by adding phosphate buffer instead of the sample in the same way as the test. The blank was prepared by adding phosphate buffer instead of the α-glucosidase. The inhibition rates (%) = $[(OD_{control} - OD_{control\ blank}) - (OD_{test} - OD_{test\ blank})]/(OD_{control} - OD_{control\ blank}) \times 100\%$. Acarbose was utilized as the positive control with an IC_{50} of 382.7 μM.

Cytotoxicity Assay. The cytotoxicity against A549 and HeLa cell lines of compounds **1**−**11** and **13**−**17** was tested using the MTT method as previously reported.[20]

Statistical Analysis. The bioactivity values were expressed as means of three independent experiments, and each was carried out in triplicate.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectral data of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +86-10-62566577. E-mail: liuhw@im.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support of the Ministry of Science and Technology of China (2014CB138304) and the National Natural Science Foundation (21472233) is acknowledged. Dr. J. Ren and Dr. W. Wang (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences) are appreciated for their help in measuring the NMR and MS data.

■ REFERENCES

(1) Mizuno, T. *Int. J. Med. Mushrooms* **1999**, *1*, 105−119.
(2) Kawagishi, H.; Ando, M.; Mizumo, T. *Tetrahedron Lett.* **1990**, *31*, 373−376.
(3) Kawagishi, H.; Ando, M.; Sakamoto, H.; Yoshida, S.; Ojima, F.; Ishiguro, Y.; Ukai, N.; Furukawa, S. *Tetrahedron Lett.* **1991**, *32*, 4561−4564.
(4) Kawagishi, H.; Ando, M.; Shinba, K.; Sakamoto, H.; Yoshida, S.; Ojima, F.; Ishiguro, Y.; Ukai, N.; Furukawa, S. *Phytochemistry* **1993**, *32*, 175−178.
(5) Kawagishi, H.; Shimada, A.; Shirai, R.; Okamoto, K.; Ojima, F.; Sakamoto, H.; Ishiguro, Y.; Furukawa, S. *Tetrahedron Lett.* **1994**, *35*, 1569−1572.
(6) Kawagishi, H.; Kojima, F.; Okamoto, K. JP 7070168, 1995.
(7) Kawagishi, H.; Simada, A.; Shizuki, K.; Mori, H.; Sakamoto, H.; Furukawa, S. *Heterocycl. Commun.* **1996**, *2*, 51−54.
(8) Kawagishi, H.; Simada, A.; Shizuki, K.; Mori, H.; Sakamoto, H.; Furukawa, S. *Tetrhedron. Lett.* **1996**, *41*, 7399−7402.
(9) Kawagishi, H.; Masui, A.; Tokuyama, S.; Nakamura, T. *Tetrahadron* **2006**, *62*, 8463−8466.
(10) Lee, E. W.; Shizuki, K.; Hosokawa, S.; Suzuki, M.; Suganuma, H.; Inakuma, T.; Kawagishi, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2402−2405.
(11) Kim, K. H.; Noh, H. J.; Choi, S. U.; Lee, K. R. *J. Antibiot.* **2012**, *65*, 575−577.
(12) Miyazawa, M.; Takahashi, T.; Horibe, I.; Ishikawa, R. *Tetrahedron* **2012**, *68*, 2007−2010.
(13) Ueda, K.; Kodani, S.; Kudo, M.; Masuno, K.; Sekiya, A.; Nagai, K.; Kawagishi, H. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 1908−1910.
(14) Rateb, M. E.; Houssen, W. E.; Harrison, W. T. A.; Deng, H.; Okoro, C. K.; Asenjo, J. A. *J. Nat. Prod.* **2011**, *74*, 1965−1971.
(15) Amagata, T.; Tanaka, M.; Yamada, T.; Doi, M.; Minoura, K.; Ohishi, H. *J. Nat. Prod.* **2007**, *70*, 1731−1740.
(16) Elisashvili, V. *Int. J. Med. Mushrooms* **2012**, *14*, 211−239.
(17) Cohen, R.; Persky, L.; Hadar, Y. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 582−594.
(18) Wang, Y. Q.; Bao, L.; Yang, X. L.; Li, L.; Li, S. F.; Hao, G.; Yao, X. S.; Wen, H. A.; Liu, H. W. *Food Chem.* **2012**, *3*, 1346−1353.
(19) Wang, S. J.; Li, Y. X.; Bao, L.; Han, J. J.; Yang, X. L.; Li, H. R.; Wang, Y. Q.; Li, S. J.; Liu, H. W. *Org. Lett.* **2012**, *14*, 3672−3675.
(20) Wang, S. J.; Bao, L.; Han, J. J.H.; Wang, Q. X.; Yang, X. L.; Wen, H. A.; Guo, L. D.; Li, S. J.; Zhao, F.; Liu, H. W. *J. Nat. Prod.* **2013**, *76*, 45−50.
(21) Wang, B. T.; Han, J. J.; Xu, W.; Chen, Y. H.; Liu, H. W. *Food Chem.* **2014**, *152*, 169−176.
(22) Allin, M. S.; Hodkinson, C. C.; Taj, N. *Synlett* **1996**, *12*, 781−782.
(23) Ogawa, K.; Nakamura, M.; Hayashi, M.; Yaginuma, S.; Yamamoto, S.; Furihata, K.; Shinya, K.; Seto, H. *J. Antibiot.* **1995**, *48*, 1396−1400.
(24) Roggo, B. E.; Petersen, F.; Sills, M.; Roesel, J. L.; Moerker, T.; Peter, H. H. *J. Antibiot.* **1996**, *49*, 13−19.

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX

(25) Li, H.; Jiang, L.; Guo, L.; Zhang, H.; Che, Y. *Bioorg. Med. Chem.* **2008**, *16*, 7894−7899.
(26) Matsumoto, K.; Nagashima, K.; Kamigauchi, T.; Kawamura, Y.; Yasuda, Y.; Ishii, K.; Uotani, N.; Sato, T.; Nakai, H.; Terui, Y.; Kikuchi, J.; Ikenisi, Y.; Yoshida, T.; Kato, T.; Itayaki, H. *J. Antibiot.* **1995**, *48*, 439−446.
(27) Wang, J. C.; Hu, S. H.; Wang, J. T.; Chen, K. S.; Chi, Y. C. *J. Agric. Food Chem.* **2005**, *85*, 641−646.
(28) Ma, K.; Han, J. J.; Bao, L.; Wei, T. Z.; Liu, H. W. *J. Nat. Prod.* **2014**, *77*, 942−947.

DOI: 10.1021/np5004388
J. Nat. Prod. XXXX, XXX, XXX−XXX