Identification and α-Glucosidase Inhibitory Activity of Meroterpenoids from *Hericium erinaceus*

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ABSTRACT

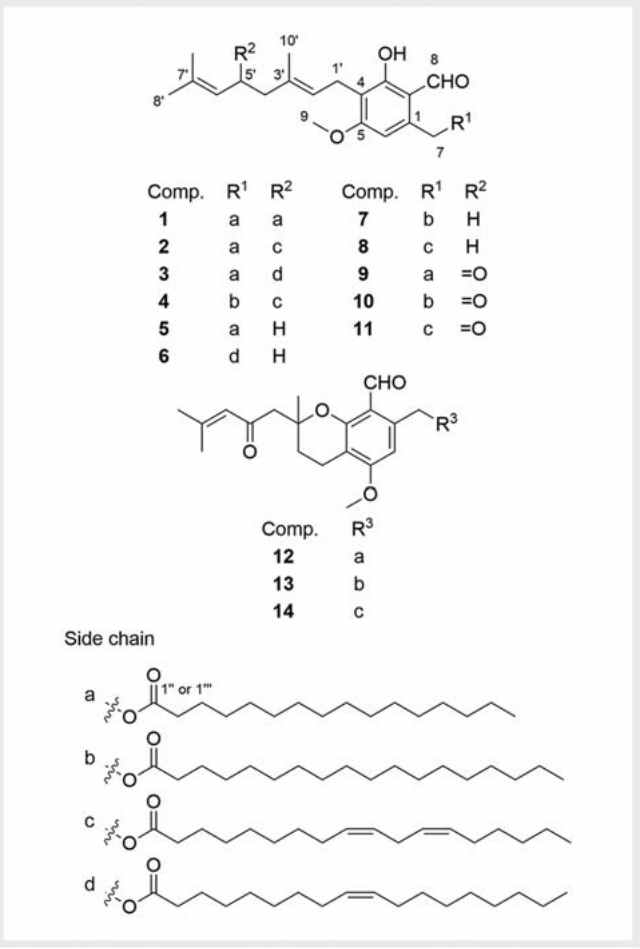
Hericium erinaceus is a very popular edible and medicinal mushroom used for the treatment of enervation and gastrointestinal diseases in Eastern Asia. Chemical investigation on the fruiting body of *Hericium erinaceus* led to the isolation of 4 new (**1**–**4**) and 10 known meroterpenoids (**5**–**14**). The structures of new compounds were determined via analysis of NMR and MS data in combination with chemical derivatization. The inhibitory activities of **1**–**14** against α-glucosidase were evaluated using *p*-nitrophenyl-α-D-glucopyranoside, sucrose, or maltose as substrate. Compounds **6, 9, 11**–**13** were demonstrated to show the α-glucosidase inhibitory activities. This work confirms the potential of *H. erinaceus* in the treatment of diabetes.

Introduction

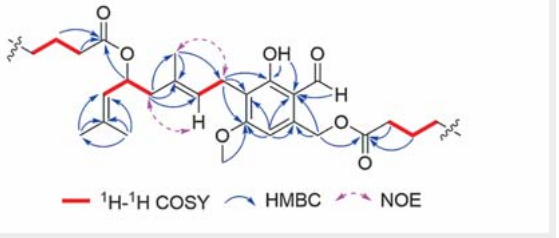
Mushrooms attract much attention of chemists and biologists due to the production of secondary metabolites with diverse structural skeletons and interesting bioactivities [1–5]. *Hericium erinaceus* (Bull.) Pers. (Hericiaceae) is a very popular edible and medicinal mushroom in Eastern Asia. The fruiting bodies of *H. erinaceus* is a traditional Chinese medicine to treat enervation and gastrointestinal diseases including dyspepsia and gastric ulcers [6]. Chemical components including sterols [5–7], meroterpenoids [8–10], diterpenes [11–14], and alkaloids [15, 16] have been identified from the fruiting bodies and mycelia of *H. erinaceus* in the past decades. The secondary metabolites from *H. erinaceus* were reported to possess various biological activities of anti-osteoporosis [17], anti-oxidation [17], cytotoxicity [18, 19], anti-*Helicobacter pylori* [20], neurite outgrowth-promotion [21], antidepression [22], and antidiabetes [23].

Diabetes is a group of metabolic diseases characterized by high blood sugar level over a prolonged period and can eventually lead to cardiovascular diseases, blindness, kidney failure, and lower limb amputation. Due to these severe complications, diabetes has become a worldwide public health issue [24]. α-Glucosidase is the key enzyme involved in catalyzing the carbohydrate hydrolysis in gut. As an important class of anti-diabetic drugs, α-glucosidase inhibitors slow down the passage of carbohydrates into the bloodstream, significantly decreasing the postprandial blood glucose level [25]. However, the currently used α-glucosidase inhibitors, including arcarbose and voglibose, encounter undesired side effects in clinics. Developing new α-glucosidase inhibitors without unwanted side effects is a great need.

Recently, a number of natural α-glucosidase inhibitors has been reported from plants and fungi [23, 26, 27]. In an early work, we isolated 9 new meroterpenoid derivatives with the α-glucosidase inhibitory activity from the mycelia extract of *H. erinaceus*



▶ **Fig. 1** Structures of compounds **1**–**14**.



▶ **Fig. 2** Key ^{1}H-^{1}H COSY, HMBC, and NOE correlations of compounds **1**–**4**.

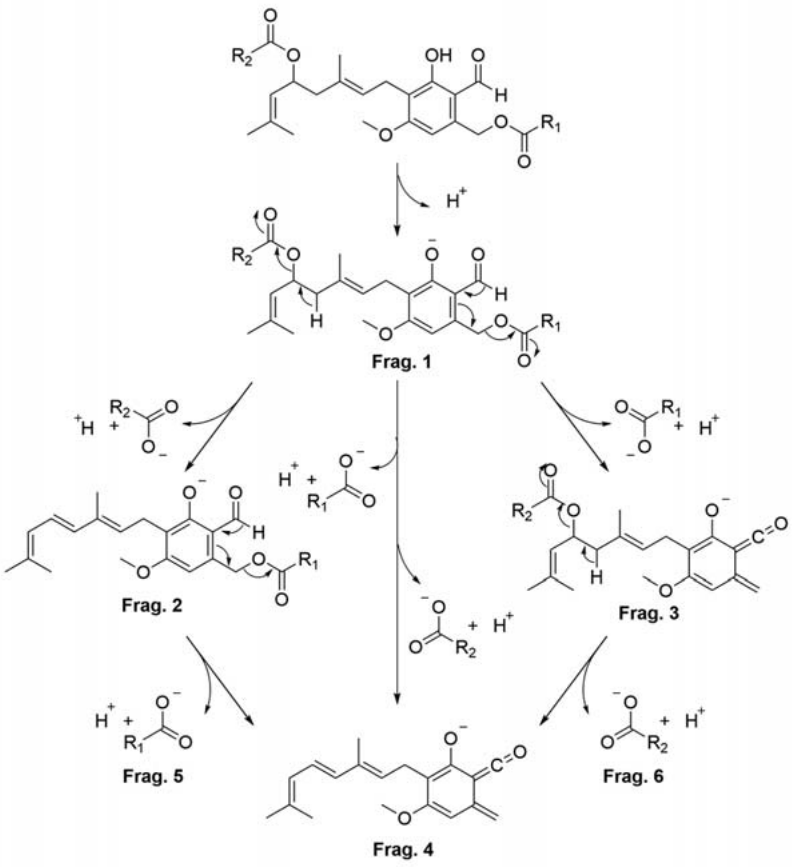
[23]. To explore secondary metabolites with α-glucosidase inhibitory activity from mushrooms, the petroleum ether extract of the fruiting bodies of *H. erinaceus* that has good α-glucosidase inhibitory activity (IC_{50} = 125 µg/mL) was put into chemical investigation. As a result, 4 new meroterpenoid esters (**1**–**4**) together with 10 known compounds (**5**–**14**) (▶ **Fig. 1**) were obtained. All compounds were evaluated for their inhibitory activities against α-glucosidase from small intestinal mucosa of rat.

Results and Discussion

The fruiting bodies of *H. erinaceus* were extracted with petroleum ether to give a brown crude extract. The extract was subjected to chromatographic separation using silica gel, ODS (octadecylsilyl), and Sephadex LH-20 chromatographic column, and semi-preparative HPLC. Four new meroterpenoid esters (**1**–**4**) and 10 known compounds (**5**–**14**) were obtained from fruiting bodies of this mushroom. The structures of known compounds hericenes A–D (**5**–**8**) and hericenones C–H (**9**–**14**) were confirmed by comparing their spectroscopic data with the literature data [8–10], respectively. The structures of new compounds were elucidated by extensive spectroscopic analysis.

The negative HRESITOFMS of hericene E (**1**) gave a pseudomolecular ion peak at 809.6274 $[M - H]^-$ (calcd. for $C_{51}H_{85}O_7$, 809.6295), suggesting a molecular formula of $C_{51}H_{86}O_7$. The ^{1}H NMR spectrum of **1** exhibited 3 singlet methyls at δ_H 1.63, 1.64 and 1.79, 2 overlapped triplet methyls at δ_H 0.88 (t, J = 6.8 Hz), 1 oxygenated singlet methyl at δ_H 3.90, 1 oxygenated singlet methylene at δ_H 5.31, 3 olefinic protons at δ_H 5.04 (d, J = 9.2 Hz), 5.19 (t, J = 7.2 Hz) and 5.59 (m), 1 singlet aromatic proton at δ_H 6.51, 1 aldehyde group at δ_H 10.10, 1 hydroxyl hydrogen at δ_H 12.34, and the highly overlapped signals at δ_H 1.24. Meanwhile, 1 aldehyde carbon at δ_C 193.04, 2 ester carbonyl carbons at δ_C 173.18 and 173.07, 10 olefinic or aromatic carbons in the range of δ_C 105.50–163.38, and 3 oxygenated carbons at δ_C 55.89, 62.91, and 69.65 were observed in the ^{13}C NMR spectrum of **1**. All above data of **1** proposed a similar structure with that of hericene A (**5**), and which was also confirmed by the ^{1}H-^{1}H COSY correlations of and H-4′–H-5′–H-6′ and H_2-1′–H-2′ together with HMBC correlations of H_2-7 with C-1, C-2, and C-6; H-6 with C-1, C-2, C-5, and C-4; H-8 with C-2; OH-3 with C-2, C-3, and C-4; H_3-9 with C-5; H_2-1′ with C-3, C-4, and C-5; H-4′ with C-2′, C-3′, and C-10′; and H_3-10′ with C-3′, C-2′, and C-1′; H_3-8 and H_3-9 with C-7′ and C-6′ (▶ **Fig. 2**). Notably, the presence of 2 triplet methyls, 2 ester carbonyl groups, and 2 oxygenated methines at δ_C 62.91 and 69.65, as well as the HMBC correlations of H-5′ with the ester carbonyl carbon at 173.18, and H_2-7 with another ester carbonyl carbon at 173.07 indicated 2 fatty acid moieties at C-5′ and C-7 in the structure of **1**. The fatty acid chains were further confirmed by analysis of its negative HRESITOF-MS/MS data. In the MS/MS spectrum of **1**, compound **1** showed a precursor ion $[M - H]^-$ at *m/z* 809.6274, a fragment ion $[M - H - C_{16}H_{32}O_2]^-$ at *m/z* 553.3885 due to the loss of a molecule of palmitic acid, a fragment ion $[M - H - C_{16}H_{32}O_2 - C_{16}H_{32}O_2]^-$ at *m/z* 297.1487 for the loss of 2 molecules of palmitic acid, and a fragment ion at *m/z* 208.9585 corresponding to the molecular formula of palmitic acid. The plausible fragments formation mechanism was shown in ▶ **Fig. 3**. The ROESY experiment showed NOE correlations (▶ **Fig. 2**) of H-1′ with H_3-10′ and H-2′ with H_2-4′, proving the *E* configuration of double bond between C-2′ and C-3′.

Hericene F (**2**) was obtained as colorless oil. Based on the negative HRESIMS data at *m/z* 833.6285 $[M - H]^-$, the molecular formula of **2** was determined to be $C_{53}H_{86}O_7$. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** showed much similarity with those of **1** except for the presence of 2 extra double bonds, which supported **2**

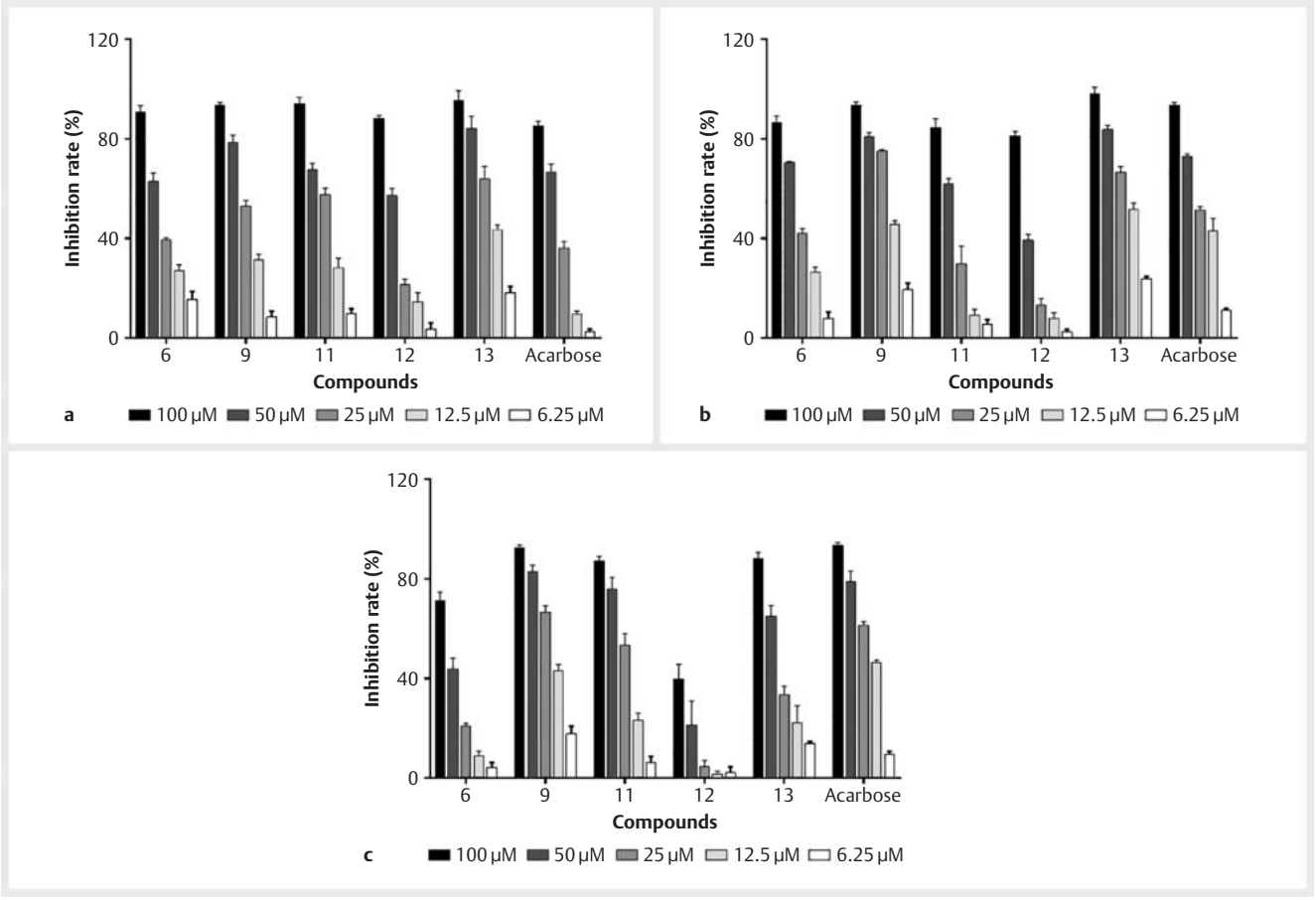


Comp.	R_1	R_2	Frag.1	Frag.2	Frag.3	Frag.4	Frag.5	Frag.6
1	$C_{15}H_{31}$	$C_{15}H_{31}$	$C_{51}H_{85}O_7^-$ *m/z* 809	$C_{35}H_{53}O_5^-$ *m/z* 553	$C_{35}H_{53}O_5^-$ *m/z* 553	$C_{19}H_{21}O_3^-$ *m/z* 297	$C_{16}H_{31}O_2^-$ *m/z* 225	$C_{16}H_{31}O_2^-$ *m/z* 225
2	$C_{15}H_{31}$	$C_{17}H_{31}$	$C_{53}H_{85}O_7^-$ *m/z* 833	$C_{35}H_{53}O_5^-$ *m/z* 553	$C_{37}H_{53}O_5^-$ *m/z* 577	$C_{19}H_{21}O_3^-$ *m/z* 297	$C_{16}H_{31}O_2^-$ *m/z* 225	$C_{18}H_{31}O_2^-$ *m/z* 279
3	$C_{15}H_{31}$	$C_{17}H_{33}$	$C_{53}H_{87}O_7^-$ *m/z* 835	$C_{35}H_{53}O_5^-$ *m/z* 553	$C_{37}H_{55}O_5^-$ *m/z* 579	$C_{19}H_{21}O_3^-$ *m/z* 297	$C_{16}H_{31}O_2^-$ *m/z* 225	$C_{18}H_{33}O_2^-$ *m/z* 281
4	$C_{17}H_{35}$	$C_{17}H_{31}$	$C_{55}H_{89}O_7^-$ *m/z* 861	$C_{37}H_{57}O_5^-$ *m/z* 581	$C_{37}H_{53}O_5^-$ *m/z* 577	$C_{19}H_{21}O_3^-$ *m/z* 297	$C_{18}H_{35}O_2^-$ *m/z* 283	$C_{18}H_{31}O_2^-$ *m/z* 279

▶ **Fig. 3** The plausible formation mechanism of MS/MS molecular ion fragments.

as a meroterpenoid incorporating 2 fatty acid moieties. A detailed analysis of the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra of **2** further confirmed the meroterpenoid skeleton and the presence of 2 fatty acid moieties. To confirm the structure of fatty acid moieties, compound **2** was hydrolyzed with alkaline followed by methyl esterification. The 2 fatty acid moieties in **2** were determined to be the linoleic acid and the palmitic acid by comparison of their retention time and MS spectrum with those of standards by GC-MS analysis. The HRESITOF-MS/MS spectrum of **2** showed a fragment ions $[M - H]^-$ at *m/z* 833.6285, $[M - H - C_{16}H_{32}O_2]^-$ at *m/z* 577.3873, $[M - H - C_{18}H_{32}O_2]^-$ at *m/z* 553.3864, $[M - H - C_{16}H_{32}O_2 - C_{18}H_{32}O_2]^-$ at *m/z* 297.1480, $[C_{18}H_{31}O_2]^-$ at *m/z* 279.2305, and $[C_{16}H_{31}O_2]^-$ at *m/z* 255.2324, which further indicated the presence of the linoleic acid and the palmitic acid (▶ **Fig. 3**). In this study, compounds **5**–**11** were obtained as major meroterpenoids from *H. erinaceus* (**Fig. S4**, Supporting Information). Taking the biosynthetic relationship between **2** and **9** into consideration, the linoleic acid moiety is supposed to be attached at C-5′, and the palmitic acid moiety is substituted at C-7.

The NMR data of hericene G (**3**) and H (**4**) were similar with those of **1** and **2**, which in combination with their corresponding HRESITOFMS data at *m/z* 835.6464 $[M - H]^-$ and 861.6597 $[M - H]^-$ indicated their structural difference in the fatty acid chains. The analysis of HRESITOF-MS/MS spectra (▶ **Fig. 3**) and GC-MS data of the alkaline hydrolysis products demonstrated the existence of the oleic acid and palmitic acid moieties in **3** and the linoleic acid and stearic acid moieties in **4**, respectively. Considering the possible biosynthetic relationship between **3** and **9**, the oleic acid moiety and the palmitic acid moiety was reasonably attached at C-5′ and C-7 in **3**, respectively. In compound **4**, the linoleic acid



▶ **Fig. 4** Inhibition assays against α-glucosidase from rat's small intestinal mucosa using *p*-nitrophenyl-α-D-glucopyranoside (**a**), sucrose (**b**) and maltose (**c**) as substrates.

moiety and the stearic acid moiety was connected at C-5′ and C-7, respectively, by considering the possible biosynthetic relationship between **4** and **10**. Compounds **1**–**4** were determined to be racemic on the basis of their small optical rotation values and the lack of significant Cotton effects in their CD spectrum.

Meroterpenoids with diverse bioactivities are key components contributing to the pharmaceutical values of *H. erinaceus*. Hericenes A–C (**5**–**7**) were reported to have nuclear factor kappa B (NF-κB) inhibitory activity [28]. Hericenones C–H (**9**–**14**) were confirmed to be stimulators of nerve growth factor (NGF)-synthesis [8, 29]. To obtain new α-glucosidase inhibitors, compounds **1**–**14** were evaluated for their inhibitory activities against α-glucosidase from the small intestinal mucosa of rats using the *p*-nitrophenyl-α-D-glucopyranoside (PNPG), sucrose, or maltose as substrate (▶ **Fig. 4**). Compounds **6, 9, 11, 12**, and **13** were found to show the α-glucosidase inhibitory activities on PNPG, sucrose, or maltose (▶ **Table 1**). Among the tested compounds, **13** showed the strongest inhibitory activities on PNPG and sucrose with the IC_{50} values of 15.2 and 12.6 μM, respectively; **9** showed the strongest inhibitory activities on maltose with the IC_{50} value of 15.3 μM. All other compound showed weak α-glucosidase inhibitory activities with IC_{50} values larger than 100 μM. The positive drugs acarbose, by contrast, showed IC_{50} values of 38.1, 20.5, and 17.1 μM for PNPG, sucrose, and maltose, respectively. Sucrose and maltose are natural substrates of α-glucosidase in gut. The α-glucosidase inhibitory effects on sucrose and maltose reflect *in vivo* activity of tested compounds. Compounds **9** and **11** showed stronger inhibitory effect than **5** and **8**, respectively, which indicates that the ketonization at C-5′ enhances the α-glucosidase inhibitory effect. In addition, the more powerful inhibitory effect of **6** than that of **5, 7**, and **8**, the stronger activity of **9** and **11** than that of **10**, and the more potent activity of **12** and **13** than that of **14** illustrated that the structure of fatty acid chain at C-7 greatly influenced the activity. On the other hand, the activity potency of **6, 9, 11**–**13** is comparable to that of erinacines D–L discovered from the culture of *H. erinaceus* [23], indicating a structural variability around C-7 and C-8 for the above activity. α-Amylase inhibitory activity was also tested for **1**–**14**. All tested compounds showed weak inhibitory activities with IC_{50} values larger than 100 μM. As the widely used α-glucosidase inhibitors in clinic, acarbose and voglibose usually cause digestive tract disorders due to their strong α-amylase inhibitory activity [25, 30]. The fact that compounds **6, 9, 11, 12**, and **13** from *H. erinaceus* showed good α-glucosidase inhibitory effect but weak α-amylase inhibitory activity supports them to be good leading compounds for new anti-diabetic drug.

▶ **Table 1** Inhibitory activity against α-glucosidase from small intestinal mucosa of rat with PNPG, sucrose, and maltose as substrates (IC_{50}, μM).

Comp.	Substrates		
	PNPG	sucrose	maltose
6	29.6 (24.4–35.9)	29.1 (24.0–35.4)	65.5 (53.4–81.0)
9	21.9 (17.2–27.9)	13.5 (10.9–16.7)	15.3 (12.6–18.4)
11	23.3 (18.4–29.5)	42.5 (31.9–57.0)	25.5 (19.8–32.8)
12	45.3 (33.1–62.6)	67.1 (48.0–95.9)	> 100
13	15.2 (12.3–18.7)	12.6 (10.6–15.1)	33.1 (26.4–41.7)
Acarbose	38.1 (28.5–51.4)	20.5 (16.5–25.4)	17.1 (13.4–21.8)

All data are presented as the mean of IC_{50} values with lower and upper 95% confidence intervals from triplicate measurement (n = 3); PNPG was the abbreviation of *p*-nitrophenyl-α-D-glucopyranoside.

In conclusion, 4 new and 10 known meroterpenoids were identified from the fruiting body of *H. erinaceus*. The structures of new compounds were determined to be the geranyl orcylaldehyde esterified by 2 fatty acids. Compounds **6, 9, 11, 12**, and **13** were demonstrated to have inhibitory activities against α-glucosidase. This research supports the mushroom of *H. erinaceus* as a potential healthy product or food supplement for diabetes patients.

Materials and Methods

General experimental procedures

NMR spectral data were obtained with Bruker Avance-500 spectrometer ($CDCl_3$, δ_H 7.26/δ_C 77.16), and HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. HR-ESI-MS and MS/MS data were measured using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument. Gas chromatograph-mass spectrometric analysis (GC-MS) was conducted on a Shimadzu GCMS-QP2010 Ultra. IR spectral data were generated by Nicolet IS5 FT-IR spectrophotometer, and UV spectra were measured via Thermo Genesys-10S UV-vis spectrophotometer. Optical rotations were recorded on an Anton Paar MCP 200 Automatic Polarimeter. CD spectra were acquired by the instrument JASCO J-815 Spectropolarimeter. OD absence was tested on Spectra Max 190 microplate reader.

Solvents used for extraction and chromatographic separation were in the analytical purity. Solvents for HPLC and GC analysis were chromatographically pure. TLC was carried out on Silica gel $HSGF_{254}$, and the spots were visualized by UV at 254 nm or spraying with 10% H_2SO_4 assisted with heating. Silica gel, octadecylsilyl (ODS, 50 μm), and Sephadex LH-20 were used for column chromatography (CC). HPLC separation was performed on an Agilent 1200 HPLC system equipped with a reversed phase C8 column (250 × 9.4 mm, 5 μm; detector: UV) with a flow rate of 2.0 mL/min.

Fungal material

Dried fruiting bodies of *H. erinaceus* bought from Fujian province were identified on the basis of morphological characteristics. A voucher specimen was deposited in the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS 388752).

Extraction and isolation

The air-dried and powdered fruiting bodies of *H. erinaceus* (0.5 kg) were supersonically extracted by petroleum ether (3 × 2 L) for 3 times (2 h each time). The organic solvent was evaporated to dryness under vacuum to afford the crude extracts (13 g). The petroleum ether extract was separated by a silica gel column chromatography (CC) using petroleum ether-ethyl acetate (P-E) in a gradient elution (100:0, 100:1, 100:2, 100:5, 100:10, 90:10, 80:20, 70:30, 60:40 *v*/*v*) to give 10 fractions (F-1 to F-10) based on the analysis of thin layer chromatography and reversed-phased high performance liquid chromatography (RP-HPLC).

Fraction F-5 (0.85 g, from P-E 100:2) was separated on an ODS CC eluted with 85% MeOH in water to get 5 subfractions (F-5-1 to F-5-5). Compounds **1–4** (5.1, 3.2, 3.5, and 5.2 mg, t_R 62.1, 59.5, 70.1, and 80.2 min) were purified from F-5-3 (204 mg) with 97% MeCN in water by RP-HPLC. Fraction F-6 (1.05 g, from P-E 100:5) was separated by Sephadex LH-20 CC eluted with MeOH-CH_2Cl_2 (50%, *v*/*v*) to get 7 subfractions (F-6-1 to F-6-7). Then F-6-3 was further subjected to an ODS CC with a gradient of MeOH in water (70–100%) to yield 6 subfractions (F-6-3-1 to F-6-3-6). Fraction F-6-3-3 eluted with 80% MeOH in water was separated by RP-HPLC with 90% MeCN in water to yield compounds **5–8** (25.4, 43.1, 33.9, and 51.2 mg, t_R 52.1, 64.3, 70.5, and 49.3 min). Fraction F-7 (2.3 g, from P-E 100:10) was separated on a Sephadex LH-20 CC eluted with MeOH-CH_2Cl_2 (50%, *v*/*v*) to yield 8 subfractions (F-7-1 to F-7-8). F-7-3 was further purified by RP-HPLC using 78% MeCN in water to give compounds **9, 11, 12**, and **14** (15.5, 31.4, 22.3, and 22.2 mg, t_R 63.3, 48.8, 73.4, and 59.3 min). Compounds **10** and **13** (31.8 and 42.9 mg, t_R 64.4 and 55.9 min) were obtained from subfraction F-7-4 by RP-HPLC using 75% MeCN in water. The physical properties, spectroscopic data of the compounds **1–4** are showed as follows.

Hericene E (**1**): colorless oil, $[\alpha]_D^{25}$ +1 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (2.34), 293 (2.28) nm; CD (*c* 1.01 × 10^{-3} M, MeOH): no significant Cotton effects; IR (neat) ν_{max} 3450, 2924, 2852, 1740, 1622, 1464, 1118 cm^{-1}; negative HRTOFMS *m/z* 809.6274 $[M - H]^-$ (calcd. for $C_{51}H_{85}O_7$, 809.6295); ^1H NMR (500 MHz, $CDCl_3$) δ_H 6.51 (s, H-6), 5.31 (s, H-7), 10.10 (s, H-8), 3.90 (s, H-9), 12.34 (s, OH-3), 3.28 (dd, *J* = 14.1, 7.2 Hz, H-1′), 3.33 (dd, *J* = 14.1, 7.2 Hz, H-1′), 5.19 (t, *J* = 7.2 Hz, H-2′), 2.27 (dd, *J* = 13.5, 7.4 Hz, H-4′), 2.08 (dd,

J = 13.5, 6.3 Hz, H-4′), 5.59 (m, H-5′), 5.04 (d, J = 9.2 Hz, H-6′), 1.64 (s, H-8′), 1.63 (s, H-9′), 1.79 (s, H-10′), 2.33 (m, H-2″), 1.61 (m, H-3″), 2.15 (m, H-2‴), 1.52 (m, H-3‴), 0.88 (t, J = 6.8 Hz, H-16″ and H-16‴), 1.24 (m, H-4″ – H-15″ and H-4‴ – H-15‴); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (C-1), 112.83 (C-2), 162.86 (C-3), 117.64 (C-4), 163.38 (C-5), 105.5 (C-6), 62.91 (C-7), 193.04 (C-8), 55.89 (C-9), 21.43 (C-1′), 124.86 (C-2′), 131.28 (C-3′), 45.31 (C-4′), 69.65 (C-5′), 123.77 (C-6′), 136.69 (C-7′), 25.61 (C-8′), 18.34 (C-9′), 16.47 (C-10′), 173.18 (C-1″), 34.23 (C-2″), 24.87 (C-3″), 173.07 (C-1‴), 34.54 (C-2‴), 24.99 (C-3‴), 31.93 (C-14″ and 14‴), 22.74 (C-15″ and 15‴), 14.13 (C-16″ and 16‴), 29.13–29.70 (C-4″–13″ and 4‴–13‴).

Hericene F (**2**): colorless oil, $[\alpha]_D^{25}$ +2 (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (2.34), 294 (2.28) nm; CD (c 7.1 × 10^{-4} M, MeOH): no significant Cotton effects; IR (neat) ν_{max} 3451, 2924, 2852, 1740, 1622, 1464, 1118 cm^{-1}; negative HRTOFMS m/z 833.6285 [M – H]$^-$ (calcd. for $C_{53}H_{85}O_7$, 833.6295); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.51 (s, H-6), 5.31 (s, H-7), 10.10 (s, H-8), 3.90 (s, H-9), 12.34 (s, OH-3), 3.28 (dd, J = 14.1, 7.2 Hz, H-1′), 3.33 (dd, J = 14.1, 7.2 Hz, H-1′), 5.19 (t, J = 7.2 Hz, H-2′), 2.27 (dd, J = 13.5, 7.4 Hz, H-4′), 2.08 (dd, J = 13.5, 6.3 Hz, H-4′), 5.59 (m, H-5′), 5.04 (d, J = 9.2 Hz, H-6′), 1.64 (s, H-8′), 1.63 (s, H-9′), 1.79 (s, H-10′), 2.33 (m, H-2″), 1.61 (m, H-3″), 2.15 (m, H-2‴), 1.52 (m, H-3‴), 2.04 (m, H-8‴ and 14‴), 5.34 (m, H-9‴, 10‴, 12‴ and 13‴), 2.76 (t, J = 6.8, H-11‴), 0.88 (m, H-16″ and 18‴), 1.24 (m, H-4″ – 15″, 4‴ – 7‴ and 15‴ – 17‴); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.65 (C-1), 112.98 (C-2), 163.01 (C-3), 117.78 (C-4), 163.53 (C-5), 105.65 (C-6), 63.06 (C-7), 193.19 (C-8), 56.04 (C-9), 21.57 (C-1′), 125.02 (C-2′), 131.42 (C-3′), 45.45 (C-4′), 69.85 (C-5′), 123.9 (C-6′), 136.85 (C-7′), 25.57 (C-8′), 18.49 (C-9′), 16.62 (C-10′), 173.32 (C-1″), 34.38 (C-2″), 25.02 (C-3″), 173.18 (C-1‴), 34.67 (C-2‴), 25.12 (C-3‴), 27.35 (C-8‴ and 14‴), 130.35, 130.22, 128.16 and 128.05 (C-9‴, 10‴, 12‴ and 13‴), 25.63 (C-11‴), 31.67 and 32.08 (C-14″ and 16‴), 22.74 and 22.85 (C-15″ and 17‴), 14.28 and 14.23 (C-16″ and 18‴), 29.23–29.85 (C-4″–13″, 4‴–7‴ and 15‴).

Hericene G (**3**): colorless oil, $[\alpha]_D^{25}$ +1 (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 211 (2.34), 293 (2.28) nm; CD (c 8.4 × 10^{-4} M, MeOH): no significant Cotton effects; IR (neat) ν_{max} 3450, 2924, 2852, 1740, 1622, 1464, 1118 cm^{-1}; negative HRTOFMS m/z 835.6464 [M – H]$^-$ (calcd. for $C_{53}H_{87}O_7$, 835.6452); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.51 (s, H-6), 5.31 (s, H-7), 10.10 (s, H-8), 3.90 (s, H-9), 12.34 (s, OH-3), 3.28 (dd, J = 14.1, 7.2 Hz, H-1′), 3.33 (dd, J = 14.1, 7.2 Hz, H-1′), 5.19 (t, J = 7.2 Hz, H-2′), 2.27 (dd, J = 13.5, 7.4 Hz, H-4′), 2.08 (dd, J = 13.5, 6.3 Hz, H-4′), 5.59 (m, H-5′), 5.04 (d, J = 9.2 Hz, H-6′), 1.64 (s, H-8′), 1.63 (s, H-9′), 1.79 (s, H-10′), 2.33 (m, H-2″), 1.61 (m, H-3″), 2.15 (m, H-2‴), 1.52 (m, H-3‴), 2.04 (m, H-8‴ and 11‴), 5.34 (m, H-9‴ and 10‴), 0.88 (m, H-16″ and 18‴), 1.24 (m, H-4″–15″, 4‴–7‴ and 12‴–17‴); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.67 (C-1), 113.04 (C-2), 163.03 (C-3), 117.81 (C-4), 163.55 (C-5), 105.76 (C-6), 63.11 (C-7), 193.27 (C-8), 56.04 (C-9), 21.56 (C-1′), 125.04 (C-2′), 131.44 (C-3′), 45.45 (C-4′), 69.92 (C-5′), 123.92 (C-6′), 136.85 (C-7′), 25.74 (C-8′), 18.48 (C-9′), 16.61 (C-10′), 173.40 (C-1″), 34.36 (C-2″), 25.01 (C-3″), 173.36 (C-1‴), 34.67 (C-2‴), 25.11 (C-3‴), 27.36 (C-8‴ and 11‴), 130.18 (C-9‴), 127.88 (C-10‴), 32.05 and 32.07 (C-14″ and 16‴), 22.83 and 22.84 (C-15″ and 17‴), 14.26 (C-16″ and 18‴), 29.20–29.90 (C-4″–13″, 4‴–7‴ and 12‴–15‴).

Hericene H (**4**): colorless oil, $[\alpha]_D^{25}$ +1 (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (2.34), 294 (2.28) nm; CD (c 1.11 × 10^{-3} M, MeOH): no significant Cotton effects; IR (neat) ν_{max} 3450, 2924, 2852, 1740, 1622, 1464, 1118 cm^{-1}; negative HRTOFMS m/z 861.6597 [M – H]$^-$ (calcd. for $C_{55}H_{89}O_7$, 861.6608); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.51 (s, H-6), 5.31 (s, H-7), 10.10 (s, H-8), 3.90 (s, H-9), 12.34 (s, OH-3), 3.28 (dd, J = 14.1, 7.2 Hz, H-1′), 3.33 (dd, J = 14.1, 7.2 Hz, H-1′), 5.19 (t, J = 7.2 Hz, H-2′), 2.27 (dd, J = 13.5, 7.4 Hz, H-4′), 2.08 (dd, J = 13.5, 6.3 Hz, H-4′), 5.59 (m, H-5′), 5.04 (d, J = 9.2 Hz, H-6′), 1.64 (s, H-8′), 1.63 (s, H-9′), 1.79 (s, H-10′), 2.33 (m, H-2″), 1.61 (m, H-3″), 2.15 (m, H-2‴), 1.52 (m, H-3‴), 2.04 (m, H-8‴ and 14‴), 5.34 (m, H-9‴, 10‴, 12‴ and 13‴), 2.76 (t, J = 6.8, H-11‴), 0.88 (m, H-18″ and 18‴), 1.24 (m, H-4″ – 17″, 4‴ – 7‴ and 15‴–17‴); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.65 (C-1), 112.98 (C-2), 163.01 (C-3), 117.78 (C-4), 163.53 (C-5), 105.65 (C-6), 63.06 (C-7), 193.19 (C-8), 56.04 (C-9), 21.57 (C-1′), 125.02 (C-2′), 131.42 (C-3′), 45.45 (C-4′), 69.85 (C-5′), 123.90 (C-6′), 136.85 (C-7′), 25.57 (C-8′), 18.49 (C-9′), 16.62 (C-10′), 173.32 (C-1″), 34.38 (C-2″), 25.02 (C-3″), 173.18 (C-1‴), 34.67 (C-2‴), 25.12 (C-3‴), 27.35 (C-8‴ and 14‴), 130.35, 130.22, 128.16, and 128.05 (C-9‴, 10‴, 12‴ and 13‴), 25.63 (C-11‴), 31.67 and 32.08 (C-16″ and 16‴), 22.74 and 22.85 (C-15″ and 17‴), 14.28 and 14.23 (C-18″ and 18‴), 29.23–29.85 (C-4″–15″, 4‴–7‴ and 15‴).

Alkaline hydrolysis reaction [31]

Each compound (1.0 mg) was dissolved in CH_2Cl_2 and hydrolyzed with 2 M NaOH/MeOH (2 mL) for 3 h at room temperature. The resulting mixture was then treated with 10 mL of HCl/MeOH under stirring for overnight. The mixture containing methyl ester of fatty acids was extracted with CH_2Cl_2 for 2 times (10 mL × 2). The organic layer was evaporated to dryness under vacuum and then dissolved in 1 mL chromatographically pure CH_2Cl_2 followed by GC-MS analysis. The samples were analyzed in split injector mode by using a fused silica capillary column Rtx-5MS (crosslinked 5% diphenyl dimethyl polysiloxane, 30 m × 0.25 mm ID × 0.25 µm) with helium (1 mL/min) as carrier. Oven temperature was programmed from 50 °C to 325 °C at a slope of 10 °C per min and then with 15 min hold. The MS was operated in EI mode (70 eV) scanning from 40 to 500 amu. The retention time of methyl oleate and methyl linoleate were at 18.80 and 18.83 min, separately.

Inhibition assays against α-glucosidase and α-amylase

Crude enzyme was extracted from rat-intestinal mucosa as previous reported with slightly modification [32]. Rats were treated according to recommendations in the Guide for the Care and Use of Laboratory Animals approved by the Committee on the Ethics of Animal Experiments of Institute of Microbiology, Chinese Academy of Sciences (IMCAS) (Permit No. APIMCAS2017023, permit date, 10th Apr. 2017). A total of 1.0 g intestinal mucosa homogenate obtained from 8-wk male SD rats was suspended in 3 mL of 0.9% saline, and the suspension was sonicated for 12 times (each 30 s) at 4 °C. After centrifugation (10 000 g) for 30 min at 4 °C, the resulting supernatant was used as the enzyme solution for the assay. Each compound or extracts dissolved in DMSO (1 µL) was

mixed with 20 µL of substrate (2.0 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG), 2.0 mM maltose, 2.0 mM sucrose, respectively), 10 µL of enzyme solution, and 69 µL of 0.1 M phosphate buffer (pH 6.9). After preincubation for 40 min, a commercial kit (GOD assay) was used to test the production of glucose from sucrose and maltose. For assays with sucrose and maltose, the absorbance was read at 550 nm under a Spectra Max 190 microplate reader. For assay with PNPG, the absorbance was measured at 405 nm for α-glucosidase inhibition. The inhibitory assay using α-amylase from porcine pancreas was performed as previously reported [33]. The blank was prepared by adding potassium phosphate buffer instead of enzyme. The control was prepared by adding potassium phosphate buffer instead of test compounds. The inhibition rates [Inhibitory rate (%) = 100 % – (OD_{test} – OD_{blank})/($OD_{control}$ – OD_{blank}) × 100 %] was calculated and plotted *vs.* test concentrations to afford the IC_{50}. All data were calculated by GraphPad Prism 5 and presented as geometric mean with 95 % confidence intervals (CI) of 3 independent experiments. Acarbose (purity ≥ 98 %) was used as a positive control.

Supporting information

The physico-chemical properties and HPLC purity analysis of the known compounds **5–14**, 1D, 2D NMR, and MS/MS spectra of compounds **1–4**, and the HPLC chromatogram together with inhibition assays activity against α-glucosidase at various concentrations are available as Supporting Information.

Contributors' Statement

Data collection: B. S. Chen, W. Z. Wang; design of the study: B. S. Chen, J. J. Han, H. W. Liu; statistical analysis: B. S. Chen, L. Bao; analysis and interpretation of the data: B. S. Chen, L. Bao, K. Ma; drafting the manuscript: B. S. Chen; critical revision of the manuscript: H. W. Liu.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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