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Isoindolinone-containing meroterpenoids with α-glucosidase inhibitory activity from mushroom *Hericium caput-medusae*

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ABSTRACT

Hericium caput-medusae is an edible and medicinal mushroom closely relative to *H. erinaceus*. According to our detailed chemical investigation, two novel isoindolinone-containing meroterpene dimers, caputmedusins A (**1**) and B (**2**), as well as nine analogues, caputmedusins C–K (**3**–**11**), were isolated from the fermentation broth of *H. caput-medusae*. Their structures were elucidated by analyses of 1D and 2D NMR spectroscopic methods. The absolute configurations of **1**–**4** were speculated based on the specific optical rotation and biogenetic consideration. The absolute configurations of **10** and **11** were rationalized by the calculation of ^{1}H NMR chemical shifts. Caputmedusins A–C (**1**–**3**) showed moderate inhibitory activity against α-glucosidase with the IC_{50} values of 39.2, 36.2 and 40.8 μM, respectively.

1. Introduction

The genus *Hericium* (basidiomycete) comprises over 12 species worldwide in the Hericiaceae family. *Hericium caput-medusae*, taxonomically related to *H. erinaceus* [1], is renowned for an edible and medicinal mushroom in oriental countries. As a traditional Chinese medicine, the crude extract of its fermentation has been made as tablets or over-the-counter (OTC) drugs to treat chronic stomach diseases in China [2]. Previous chemical study of this mushroom highlighted on the isolation of water-soluble polysaccharides with potential biological evaluation in the aqueous fraction [3,4]. Nevertheless, there is poor understanding about secondary metabolites of this mushroom in the organic fraction.

Isoindolinone derivatives are a large group of heterocyclic alkaloids which have been embraced unabatedly by organic chemists in last two decades for their preeminent structures and versatile biological activities [5]. Among them, more than 26 isolates possessing isoindolinone scaffold have been uncovered from the genus *Hericium* (Fig. S1). According to our detailed chemical study on the fermentation broth of *H. caput-medusae*, two novel isoindolinone-containing meroterpene dimers, caputmedusins A (**1**) and B (**2**), a possible biosynthetic intermediate caputmedusaldehyde (details of structure elucidation in the supplementary data), as well as nine new analogues, caputmedusins C–K (**3**–**11**), were obtained (Fig. 1). Their structures were elucidated by analyses of 1D and 2D NMR spectroscopic methods. The absolute configurations of **1**–**4** were speculated based on the specific optical rotation, and biogenetic consideration. The absolute configurations of **10** and **11** were rationalized by the calculation of ^{1}H NMR chemical shifts. All isolates were evaluated for their inhibitory activities against α-glucosidase.

2. Result and discussion

2.1. Structure elucidation

Compound **1** was obtained as yellow gum with the value of specific optical rotation $[\alpha]_D^{20}$ − 20.7 (*c* 0.24, MeOH). Its molecular formula $C_{42}H_{50}O_{12}N_2$ with nineteen double-bond equivalents was deduced by sodium adduct (+)-HRESIMS ion peak at *m/z* 797.3259 $[M + Na]^+$ (calcd for 797.3256). The ^{1}H NMR spectrum (Table 1) revealed the presence of characteristic protons at δ_H 6.76 (H-7′, s), 6.72 (H-13, H-13′, t, J = 7.6 Hz), 6.71 (H-7, s), 5.28 (H-9, H-9′, t, J = 7.5 Hz), 1.80 (H_3-16, H_3-16′, s), 1.74 (H_3-17, H_3-17′, s). In accord with the molecular formula, forty two carbon signals were resolved in its ^{13}C NMR spectrum (Table 1) including four methyls, twelve methylenes (including three nitrogenated carbons at 49.2, 46.4 and 43.3), seven methines (six olefinic/aromatic carbons at 144.0, 144.0, 124.5, 124.5, 102.1, 102.0 and one nitrogenated carbon at 55.2), and nineteen sp^2 nonprotonated

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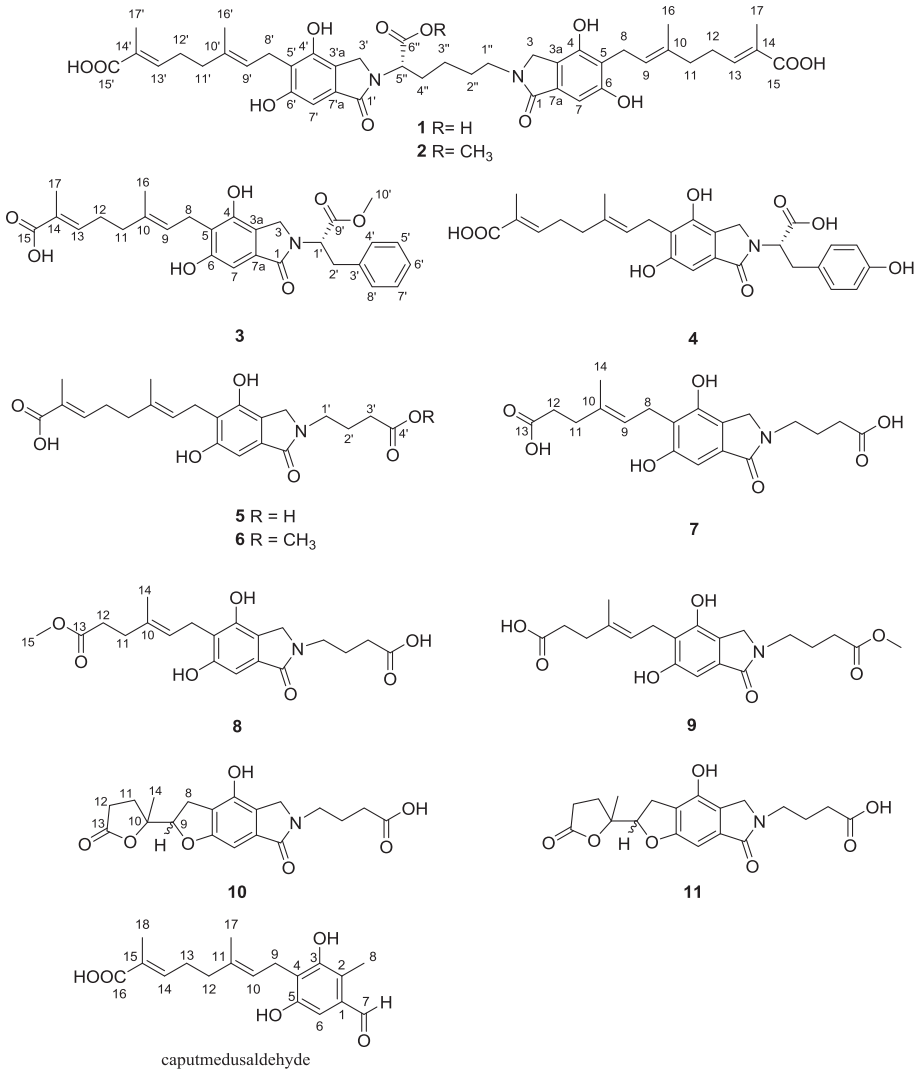

Fig. 1. Structures of **1–11** and caputmedusaldehyde from *Hericium caput-medusae*.

carbons (two amide carbonyls at 172.4 and 171.9, three carboxyls at 174.5, 171.5 and 171.5 and fourteen olefinic/aromatic carbons). The aforementioned functional groups accounted for 15 out of 19 degrees of unsaturation and thus required four rings in the molecule. Careful alignment of the ^{13}C NMR data indicated that the predominant carbon signals appeared as pairs shown in Table 1, revealing that **1** was likely to be an isoindolinone-containing meroterpene dimer.

The planar structure of **1** was constructed by 2D NMR analyses (including ^{1}H-^{1}H COSY, HSQC, HMBC). Five isolated spin systems were established by the ^{1}H-^{1}H COSY correlations (Fig. 2). These isolated spin systems and nonprotonated carbons were closely connected by the following key HMBC connections. The HMBC cross peaks from H-7 to C-1, C-3a, C-4, C-5 and C-7a; from H_2-3 to C-3a, C-4, C-7a and C-1″ established the bicyclic isoindolinone scaffold. Additionally, the HMBC correlations of H_2-8/C-4, C-5, C-6, C-9 and C-10 offered solid evidence that the side chain containing a geranyl group in part A was fixed to C-5. In parallel, the HMBC cross peaks from H_2-3′ to C-3′a, C-4′, C-7′a and C-5″, and from H-7′ to C-1′, C-3′a, C-4′, C-5′ and C-7′a also established the identical bicyclic isoindolinone skeleton, and the HMBC correlations of H_2-8′/C-4′, C-5′, C-6′, C-9′ and C-10′ constructed part B of **1**. The part C was elucidated by the HMBC correlations as well as ^{1}H-^{1}H COSY as drawn in Fig. 2. The two parts A and B were finally linked via the part C by the key HMBC correlations of H_2-1″/C-1 and C-3; H-5″/C-1′, C-3′, C-4″ and C-6″. Overall, the analyses strongly hinted that **1** had a dimer of isoindolinone derivative. The ROESY correlations between H_3-17 and H_2-12, H_3-16, H_2-8, H_3-17′ and H_2-12′ and H_3-16′ and H_2-8′ (Fig. 2) suggested the *E* configuration for these four double bonds. Above all, 2D NMR spectra and HRESIMS analyses verified the planar structure and relative configuration of **1**. Given that only one chiral center at C-5″ appeared in **1**, it was reliable to determine the absolute configuration by comparing values of specific optical rotation. The specific optical rotation of **1** was $[\alpha]_D^{20}$ − 20.7 (*c* 0.24, MeOH), which was consistent with the known counterpart monomer erinacerin K, $[\alpha]_D^{25}$ − 65.4 (*c* 1.20, MeOH), indicating the same absolute configuration for the chiral center at C-5″ for these two compounds. Additionally, the sign of specific optical rotation was positive for the opposite absolute configuration of congener erinacerin K [6]. These results supported that stereochemistry at C-5′ was *S*. The structure of **1**, caputmedusin A, was thus unambiguously characterized as shown (Fig. 1).

Compound **2**, optically active and yellow gum with the $[\alpha]_D^{20}$ − 21.5 (*c* 0.09, MeOH), had a molecular formula of $C_{43}H_{52}O_{12}N_2$ assigned by the deprotonated molecular ion peak for $[M-H]^-$ at *m/z* 787.3454 (calcd for 787.3447) on the basis of HRESIMS with 38 indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR of **2** (Table 1) were highly analogous to those of **1**, except for the presence of an additional methyl (δ_H 3.70, 3H, s; δ_C 51.3). The HMBC correlation from H_3-7″ to C-6″ indicated the linkage of a methoxyl group at C-6″. Based on the ROESY spectrum and value of specific optical rotation, absolute configuration of **2** was assigned to be identical

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1** and **2** (δ in ppm).

No.	1		2	
	δ_H[a] (J in Hz)	δ_C[b]	δ_H[a] (J in Hz)	δ_C[b]
1		171.9		169.9
3	4.26 s	49.2	4.28 s	47.6
3a		131.9		130.2
4		151.6		150.1
5		121.9		120.4
6		158.1		156.5
7	6.71 s	102.0	6.74 s	100.4
7a		120.5		119.3
8	3.41 d (7.5)	23.8	3.41 d (7.7)	22.1
9	5.28 t (7.5)	124.5	5.3 t (7.7)	122.8
10		134.9		133.2
11	2.07 t (7.0)	39.5	2.09 t (7.0)	37.9
12	2.26 m	28.5	2.28 m	26.8
13	6.72 t (7.6)	144.0	6.72 t (7.5)	142.0
14		128.8		127.4
15		171.5		170.3
16	1.80 s	16.5	1.81 s	14.8
17	1.74 s	12.6	1.75 s	10.9
1′		172.4		171.4
3′	4.28 d (17.0)	46.4	4.29 d (16.6)	44.7
	4.45 d (17.0)		4.20 d (16.6)	
3′a		131.2		129.4
4′		151.7		150.0
5′		121.5		119.8
6′		158.1		156.6
7′	6.76 s	102.1	6.71 s	100.3
7′a		121.0		118.8
8′	3.41 d (7.5)	23.8	3.42 d (7.2)	22.1
9′	5.28 t (7.5)	124.5	5.28 t (7.2)	122.9
10′		135.0		133.4
11′	2.07 t (7.0)	39.5	2.09 t (7.0)	37.9
12′	2.26 m	28.5	2.28 m	26.9
13′	6.72 t (7.6)	144.0	6.72 t (7.5)	142.1
14′		128.8		127.4
15′		171.5		170.3
16′	1.80 s	16.5	1.82 s	14.7
17′	1.74 s	12.6	1.75 s	10.9
1″	3.55 m	43.3	3.58 m	41.5
2″	1.68 m	28.7	1.71 m	27.0
	1.78 m		1.79 m	
3″	1.34 m	24.7	1.35 m	22.8
4″	2.00 m	30.2	2.02 m	28.4
	2.16 m		2.15 m	
5″	4.91 dd (11.1,4.7)	55.2	4.94 dd (11.1,4.8)	53.5
6″		174.5		170.6
−OCH_3			3.70 s	51.3

[a] Measured in methanol-d_4 at 600 MHz.
[b] Measured in methanol-d_4 at 150 MHz.

with those of **1**. Compound **2**, caputmedusin B, was thereby elucidated.

A plausible biosynthetic pathway for the formation of **1** and **2** is briefly postulated as follows (Fig. 3) on the basis of the intermediate caputmedusaldehyde and literature investigation [7,8]. The trace amount of caputmedusaldehyde was co-isolated from *H. caput-medusae*. This caputmedusaldehyde was derived from terpenoid pathway and polyketide pathway. In the following biosynthetic process, one molecule of natural amino acid L-lysine is responsible for the linkage of two molecules of intermediate III to materialize the final products **1** and **2**.

Although the discovery of **1** and **2** is a blockbuster, it also possessed a question whether they are natural products or handling artifacts. To clarify this, the analyses of EtOAc extract were performed on LC-MS. The results (Fig. S31) clearly revealed the existence of an ion peak at m/z 797 ($[M + Na]^+$, t_R 16.5 min), 813 ($[M + K]^+$, t_R 16.5 min) and the other ion peak at m/z 811 ($[M + Na]^+$, t_R 20.0 min), 827 ($[M + K]^+$, t_R 20.0 min), indicating the natural occurrences of **1** and **2**, respectively.

Compound **3**, optically active yellow oil, was assigned as the molecular formula $C_{28}H_{31}NO_7$ by the HRESIMS ion at m/z 494.2188 $[M + H]^+$ (calcd for 494.2173). The 1D NMR data (Table 2) and HSQC spectrum revealed the presence of a 1,2,3,4,5-substituted benzene ring (δ_H 6.73 and δ_C 101.9, 120.8, 131.0, 151.5, 121.9, 158.1) and mono-substituted benzene ring (δ_H 7.15–7.21, 5H and δ_C 138.2, 129.7, 129.7, 127.9, 129.7129.7), three methyls at δ_H 1.80 (s, H_3-16), 1.76 (s, H_3-17) and 3.74 (s, H_3-10′), two olefinic protons at δ_H 5.27 (br t, J = 7.2 Hz, H-9), 6.72 (br t, J = 7.2 Hz, H-13). The analyses of the ^{13}C NMR data disclosed typical carbons including three carbonyls (δ_C 171.7, 171.8, 172.5), five methylenes and one methine (nitrogenated carbon). The ^{1}H NMR and ^{13}C NMR spectra were quite similar to those of erinacerin K. The ^{1}H-^{1}H COSY correlations (Fig. 4) of H-13/H_2-12/H_2-11, $H_2$8/H-9 with the HMBC correlations of H-8/C-9, C-10, H_3-16/C-8, C-9, C-10, C-11 and H_3-17/C-13, C-14, C-15 (δ_C 171.8) constructed a geranyl group featuring the oxidization of a terminal methyl to carboxylic acid. This geranyl group was linked to C-5 via the HMBC correlations from H-8 to C-4, C-5 and C-6. The ROESY correlations of H_3-17/H-12, H_3-16/H-8, H_2-11/H-9 assigned the E configuration for the $\Delta^{9,10}$ double bond and $\Delta^{13,14}$ double bond. Furthermore, the absolute configuration of **3** at C-1′ was proposed as S by comparing values of specific optical rotation of **3** ($[\alpha]_D^{20}$ − 87.0 (c 0.18, MeOH)) with erinacerin K ($[\alpha]_D^{20}$ − 65.4 (c 1.20, MeOH)). Thus, the structure of **3** was established as caputmedusin C.

The molecular formula of compound **4** was established as $C_{27}H_{29}NO_8$ by HRESIMS ion at m/z 496.1965 $[M + H]^+$ (calcd for 496.1966). Detailed analyses of 1D NMR data and 2D NMR spectra showed that it was similar to those of **3**, except for nitrogen-linked moiety (N-2). An acetyl group for the 9′-substituent in **3** was subjected to hydrolyzation and a hydroxy group was added to C-6′ to form **4**.

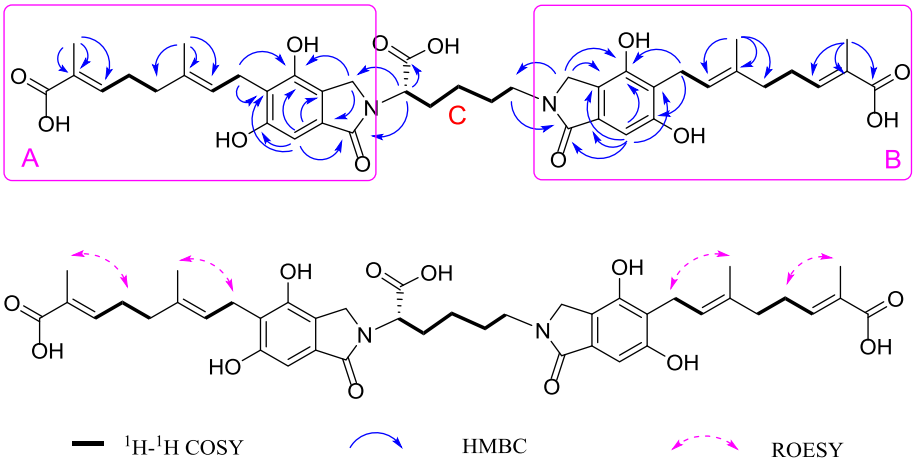

Fig. 2. Characteristic COSY, HMBC and ROESY correlations of compound **1**.

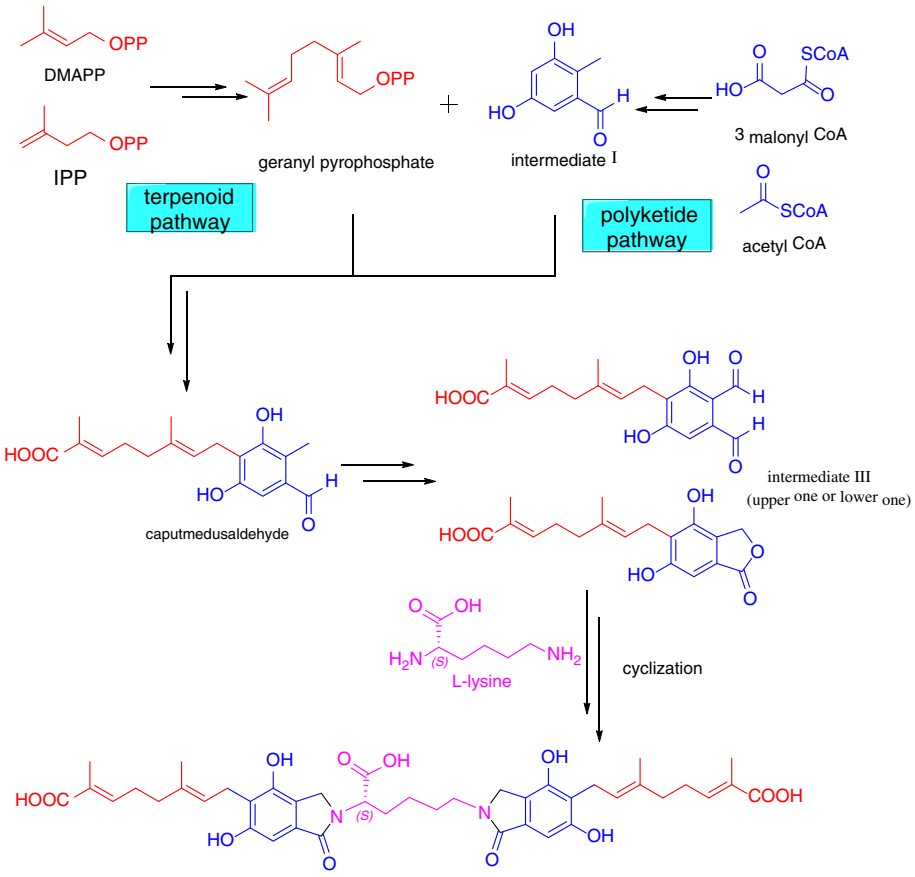

Fig. 3. Hypothetical biosynthetic pathway of **1**.

These assignments were supported by chemical shift C-6′ (δ_C 157.2) and HRESIMS analysis. Likewise, ROESY correlations of H_3-17/H-12, H_3-16/H_2-8, H_2-11/H-9 determined the *E* configuration for the $\Delta^{9,10}$ double bond and $\Delta^{13,14}$ double bond. The absolute configuration of **4** at C-1′ was speculated as *S* by comparing specific optical rotation values of **4** ($[\alpha]_D^{20}$ − 84.0 (*c* 1.0, MeOH)) with erinacerin K ($[\alpha]_D^{20}$ − 65.4 (*c* 1.2, MeOH)). Therefore, compound **4** was established and named caputmedusin D.

Compounds **5** and **6** were isolated as yellow oil and yellow powder, respectively. Their molecular formulas $C_{22}H_{27}NO_7$ and $C_{23}H_{29}NO_7$ were deduced based on the HRESIMS ions at *m/z* 418.1871 $[M + H]^+$ (calcd for 418.1860) and at *m/z* 430.1861 $[M - H]^-$ (calcd for 430.1871), respectively. Analyses of ^{1}H NMR and ^{13}C NMR data revealed that compound **5** shared the same skeleton with that of **3**, except for the nitrogen-linked moiety. Thoroughly, the COSY correlation (Fig. 4) of H_2-1′/H_2-2′/H_2-3′, as well as the HMBC correlations of H_2-2′/C-4′ (δ_C 176.9), established a 3′-carboxypropyl group, which was attached to N-2 by HMBC correlations from H_2-1′ to C-1 and C-3. Comparing the NMR data of **5** with those of **6**, it was deduced that compound **5** was subjected to the esterification at C-4′ to form **6**. This deduction was verified by the HMBC cross-peak from H_3-5′ (δ_H 3.57) to C-4′ (δ_C 173.8). The *E* configurations for the $\Delta^{9,10}$ double bond and $\Delta^{13,14}$ double bond of both **5** and **6** were refined by ROESY correlations of H_3-17/H_2-12, H_3-16/H_2-8, H_2-11/H-9. Hence, the structures of compounds **5** and **6** were constructed and given trivial names caputmedusins E and F, respectively.

Compound **7** was purified as yellow oil. It possessed the molecular formula of $C_{19}H_{23}NO_7$ deduced by the HRESIMS ion at *m/z* 376.1402 $[M - H]^-$ (calcd for 376.1402). The ^{1}H and ^{13}C NMR data of **7** resembled that of **5**, except for degradation of $\Delta^{13,14}$ double bond to form carboxylic acid. This deduction was confirmed by the COSY correlations of H_2-8 (δ_H 3.41)/H-9 (δ_H 5.30) and H_2-11 (δ_H 2.25)/H_2-12 (δ_H 2.35), with the aid of HMBC correlations of H_2-8/C-4, C-6, C-10, H_3-14/C-9, C-10, C-11, H_2-11/C-13, H_2-12/C-13. The ROESY correlation of H_3-14/H_2-8 and H_2-11/H-9 supported the *E* configuration of the $\Delta^{9,10}$ double bond. Compound **7** was, therefore, elucidated as caputmedusin G.

Compounds **8** and **9** were isolated as yellow powder with identical molecular formula $C_{20}H_{25}NO_7$ as determined by HRESIMS. The ^{1}H and ^{13}C NMR data of these two compounds were closely similar to **7**, except for the presence of an extra methoxyl group at C-13 in **8** and one methoxyl group at C-4′ in **9**, respectively. The HMBC cross-peak of H_3-15 (δ_H 3.55) with C-13 (δ_C 175.8) in compound **8** substantiated the connection of the methyoxyl group. In parallel, the location of the methyoxyl group in compound **9** was revealed by the HMBC correlation of H_3-5′ (δ_H 3.58) with C-4′ (δ_C 175.2). Moreover, the *E* configuration of the $\Delta^{9,10}$ double bond was also confirmed in compounds **8** and **9** via ROESY spectra. Accordingly, the structures of **8** and **9** were deduced as caputmedusins H and I, respectively.

Compounds **10** and **11** were isolated as optically active, yellow powder, which had the identical molecular formulas $C_{19}H_{21}NO_7$ as determined by HRESIMS measurements of the sodium adduct ion at *m/z* 398.1207 $[M + Na]^+$ (calcd for 398.1210). Analyses of the NMR data of **10** suggested that it had an isoindolinone skeleton. The HMBC correlations of H_2-8/C-4, C-6, H-9/C-6 along with the COSY correlation of H_2-8/H-9 established a 6,9-ether bridge featuring a five-membered ring. The HMBC correlations of H_3-14/C-11, H_2-11/C-13 and H_2-12/C-13 (δ_C 179.6) and COSY correlation of H_2-11/H_2-12 revealed the presence of a γ-lactone [9], which was attached to C-9 as supported by key HMBC correlations from H-9 to C-10 (δ_C 88.3) and C-11. In addition, the ^{1}H-^{1}H COSY correlations of H_2-1′/ H_2-2′/ H_2-3′, as well as the HMBC correlations of H_2-2′/C-4′ (δ_C 177.9), established a 3′-carboxypropyl group. The group was linked to the N-2 as supported by HMBC correlations from H-1′ to C-1 and C-3. Overall analyses confirmed the planar structure as shown in Fig. 1. The ROESY correlation between H_3-

Table 2
^{1}H and ^{13}C NMR spectroscopic data of **3–5** (δ in ppm).

No.	3		4		5	
	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[a]	δ_H[b] (*J* in Hz)
1	171.7		171.9		171.6	
3	47.2	4.30 d (16.5) 4.24 d (16.5)	47.1	4.36 d (16.7) 4.29 d (16.7)	49.3	4.31 s
3a	131.0		120.9		120.5	
4	151.5		151.5		151.6	
5	121.9		121.8		121.4	
6	158.1		158.0		158.0	
7	101.9	6.68 s	102.0	6.70 s	102.0	6.74 s
7a	120.8		131.2		131.8	
8	23.6	3.39 d (7.2)	23.7	3.41 d (7.0)	23.7	3.42 d (7.2)
9	124.2	5.27 br t (7.2)	124.3	5.28 br t (7.0)	124.4	5.28 t (7.2)
10	135.0		135.0		134.8	
11	39.4	2.07 t (7.6)	39.4	2.07 t (7.6)	39.4	2.07 t (7.6)
12	28.4	2.26 m	28.4	2.26 m	28.3	2.26 m
13	143.6	6.72 br t (7.2)	143.9	6.74 br t (7.6)	143.9	6.72 br t (7.5)
14	129.1		128.7		128.8	
15	171.8		171.8		171.8	
16	16.3	1.80 s	16.4	1.80 s	16.4	1.80 s
17	12.5	1.76 s	12.4	1.76 s	12.5	1.74 s
1′	57.1	5.18 dd (11.0, 5.3)	57.2	5.12 dd (11.2, 4.9)	43.1	3.61 t (7.1)
2′	36.4	3.21 dd (14.5, 11.0) 3.45 dd (14.5, 5.3)	35.7	3.39 dd (14.7, 4.9) 3.12 dd (14.7, 11.2)	24.8	1.96 m
3′	138.2		129.1		32.2	2.35 t (7.3)
4′	129.7	7.21 overlapped	130.7	7.03 br d (8.6)	176.9	
5′	129.7	7.21 overlapped	116.4	6.66 br d (8.6)		
6′	127.9	7.15 m	157.2			
7′	129.7	7.21 overlapped	116.4	6.66 br d (8.6)		
8′	129.7	7.21 overlapped	130.7	7.03 br d (8.6)		
9′	172.5		174.1			
10′	53.0	3.74 s				

[a] Measured in methanol-d_4 at 125 MHz.
[b] Measured in methanol-d_4 at 500 MHz.

14 and H-9 suggested that they were cofacial. In parallel, the planar structure of compound **11** was assigned as same as to that of **10** based on highly similar 2D NMR spectra. Reinspection of chemical shifts of **10** and **11** uncovered the subtle variations of chemical shits of H_2-8 ($\Delta\delta_H$ 0.07, 0.18), H-9 ($\Delta\delta_H$ 0.07), H_2-11 ($\Delta\delta_H$ 0.28, 0.10). These variations of chemical shifts, as well as the values of specific optical rotation $[\alpha]_D^{20}$ − 123.3 (*c* 0.08, MeOH) for **10** and $[\alpha]_D^{20}$ + 65.3 (*c* 0.09, MeOH) for **11**, suggested that compounds **10** and **11** should be a pair of diastereoisomers. Given that cotton effects in the circular dichroism (CD) spectra of **10** and **11** were extraordinarily weak (Figs. S96 and S105), the absolute configurations of **10** and **11** at C-9 and C-10 were tentatively rationalized by calculations of ^{1}H NMR chemical shifts [10,11]. Compounds **10** or **11** have four possible candidates *RR* (a possible candidate of 9*R*, 10*R* configurations for compound **10** or **11**), *SS* (a possible candidate of 9*S*, 10*S* configurations for compound **10** or **11**), *RS* (a possible candidate of 9*R*, 10*S* configurations for compound **10** or **11**), *SR* (a possible candidate of 9*S*,10*R* configurations for compound **10** or **11**) (Fig. S106). Predominant conformers of four possible

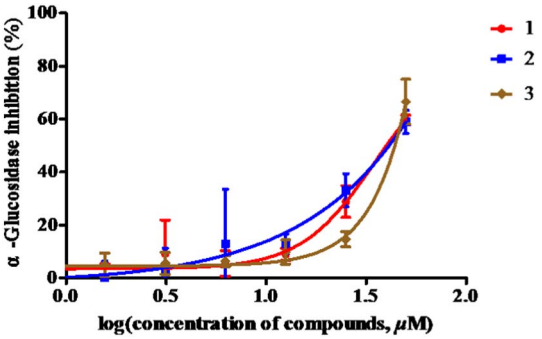

Fig. 5. α-Glucosidase inhibitory activities of compounds **1** and **2** at different concentrations (μM).

candidates (*RR* and *RS*) were calculated the ^{1}H NMR data with Gaussian 09 software package [12]. The regression analysis of calculated versus experimental ^{1}H NMR chemical shifts of **10** and **11** were performed (Table S2). The calculated NMR data of the candidate *SR* were exclusively closer to the experimental data of both **10** and **11** as weighed by the linear correlation coefficients (R^2). This cue evidently revealed that the absolute configurations of compounds **10** and **11** at C-9 and C-10 were refutable via the calculation of NMR data and further work is needed to ultimately pinpoint the absolute configurations of compounds **10** and **11**. The structures of **10** and **11** were thus named caputmedusins *J* and K, respectively.

2.2. Biological activity assays

Compounds **1–11** were evaluated for their hypoglycemic activity utilizing an in vitro α-glucosidase inhibition assay. Compounds **1–3** showed moderate inhibitory (Fig. 5) against α-glucosidase with the IC_{50} values of 39.2, 36.2, 40.8 μM, respectively. Other compounds (**4–11**) showed no inhibition against α-glucosidase.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV/Vis spectra were obtained using a Shimadzu UV2401PC spectrometer (Shimadzu, Kyoto, Japan). CD spectra were tested on an Applied Photophysics Chirascan Circular Dichroism Spectrometer (Applied Photophysics Limited, Leatherhead, Surrey, UK). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA) with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker Avance III 500 MHz and Bruker Avance III 600 MHz spectrometers (Bruker Biospin GmbH, Karlsruhe, Germany). HRESIMS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., P. R. China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for chromatography colum. MPLC was performed on a Büchi Sepacore System equipped with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Switzerland), and

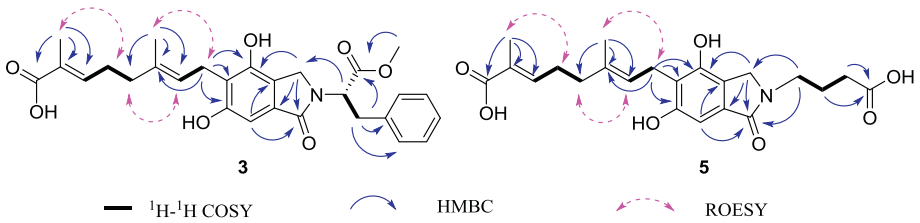

Fig. 4. Characteristic COSY, HMBC and ROESY correlations of compounds **3** and **5**.

columns packed with Chromatorex C18 (40–75 mm, Fuji Silysia Chemical Ltd., Japan). Preparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with two types of Zorbax SB-C18 columns (9.4 mm × 150 mm and 21.2 mm × 150 mm, particle size 5 mm).

3.2. Fungal material

The fungus *H. caput-medusae* was collected from Yunhe County, Zhejiang province, China, in July 2007. The fungus was identified by Prof. Mu Zang at the Kunming Institute of Botany. The fungal strain was isolated from the fruiting bodies and cultivated in potato dextrose agar (PDA) plate. A voucher specimen (HFG0701902) was deposited at the Herbarium of Kunming Institute of Botany.

3.3. Extraction and isolation

An *H. caput-medusae* agar slant was transferred onto a PDA plate and incubated at 22 °C for 7 days. The agar plugs were cut into small pieces (approximately 0.5 × 0.5 × 0.5 cm^3), then strain was inoculated into 170 Erlenmeyer flasks, respectively. Each 500 mL Erlenmeyer flask contained 300 mL modified PDA medium (potato 200.0 g; glucose 20.0 g; KH_2PO_4 3.0 g; $MgSO_4$ 1.5 g and citric acid 0.1 g in 1 L deionized water, pH was adjusted to 6.5 before autoclaving). Fermentation was carried out in two rotary shakers at 22 °C and 150 rpm for 25 days.

After the incubation, the entire culture was extracted with EtOAc. The crude extract (45 g) was fractionated by MPLC with a stepwise gradient MeOH/H_2O (*v*/v 0:100, 20:80, 40:60, 50:50, 60:40, 70:30, 80:20, 100:0) to give eight fractions (Fr.A-I). Fr.E (9.5 g) was subjected to MPLC with isocratic elution (MeOH/H_2O, 60:40) to obtain six subfractions (E1-E6) based on TLC analyses. Subfractions E5 and E7 were separated on Sephadex LH-20 (MeOH) to give three fractions (E5a, E5b and E5c), three fractions (E7a, E7b, E7c), respectively. Fr.E5b and Fr.E7c were purified by silica gel chromatography column with a petroleum ether-acetone gradient solvent system (*v*/v, 4:1-1:1) to remove impurities. Compounds **1** (15 mg, t_R 8.0 min), **2** (10 mg, t_R 11.8 min) and caputmedusaldehyde (1.2 mg, t_R 12.7 min) were obtained from fraction E5b by prep-HPLC using a gradient elution (MeCN/H_2O 35%–45%, 7 mL·min^{-1}, 20 min). In the same way, compound **3** (9.6 mg, t_R 11.0 min) was obtained from fraction E7c. Fr.C (15.6 g) was subjected to MPLC with isocratic elution (MeOH/H_2O, 40:60) to obtain six subfractions (C1-C7) based on TLC analyses. Subfraction C7 was separated by Sephadex LH-20 (MeOH) to afford four fractions (C7a, C7b, C7c, C7d). Compounds **9** (17.2 mg, t_R 15.5 min) and **8** (11.3 mg, t_R 19.3 min) were obtained from fraction C7b by prep-HPLC using a gradient elution (MeCN/H_2O 15%–30%, 7 mL·min^{-1}, 30 min). Compound **7** (8.6 mg, t_R 16.0 min) was obtained from fraction C7d by prep-HPLC using a gradient elution (MeCN/H_2O 13%–28%, 7 mL·min^{-1}, 30 min). Likewise, Fr. D (7.8 g) was subjected to MPLC with isocratic elution (MeOH/H_2O, 50:50) to obtain five subfractions (D1-D5) based on TLC analyses. Fraction D3 was isolated by silica gel chromatography column with a petroleum ether-acetone gradient solvent system (*v*/v, 4:1-1:1) to give five subfractions (D3a-D3e). Compounds **4** (12.1 mg, t_R 9.3 min), **5** (7.8 mg, t_R 7.8 min), and **6** (16.4 mg, t_R 11.6 min) were isolated from D3e, D3c and D3a respectively by prep-HPLC using a gradient elution (MeCN/H_2O 32%–42%, 14 mL·min^{-1}, 20 min).

Caputmedusin A (1): yellow gum, $[\alpha]_D^{20}$ − 20.7° (*c* 0.24, MeOH). IR (KBr) υ_{max} 3417, 2929, 2870, 2654, 1665, 1608, 1464, 1420, 1384, 1348 cm^{-1}. UV (MeOH) λ_{max} (log ε) 215.0 (4.85) nm. ^1H and ^{13}C NMR data, see Table 1; positive ESIMS: *m/z* 797 $[M + Na]^+$; HRESIMS: *m/z* 797.3259 $[M + Na]^+$ (calcd for $C_{42}H_{50}O_{12}N_2Na$, 797.3256).

Caputmedusin B (2): yellow gum, $[\alpha]_D^{20}$ − 21.5° (*c* 0.09, MeOH). IR (KBr) υ_{max} 3422, 2928, 2867, 1738, 1666, 1611, 1549, 1464, 1418, 1383, 1350, 1240, 1174, 1056 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (5.02) nm. ^1H and ^{13}C NMR data, see Table 1; negative ESIMS: *m/z* 787 $[M - H]^-$; HRESIMS: *m/z* 787.3454 $[M - H]^-$ (calcd for $C_{43}H_{51}O_{12}N_2$, 787.3447).

Caputmedusin C (3): yellow oil, $[\alpha]_D^{20}$ − 87.0° (*c* 0.18, MeOH). IR (KBr) υ_{max} 3419, 3031, 2927, 2952, 2860, 1741, 1669, 1610, 1462, 1354, 1216, 1175, 1061 cm^{-1}. UV (MeOH) λ_{max} (log ε) 213.4 (4.65) nm. ^1H and ^{13}C NMR data, see Table 2; positive ESIMS: *m/z* 494 $[M + H]^+$; HRESIMS: *m/z* 494.2188 $[M + H]^+$ (calcd for $C_{28}H_{32}O_7N$, 494.2173).

Caputmedusin D (4): yellow oil, $[\alpha]_D^{20}$ − 84.0° (*c* 1.02, MeOH). IR (KBr) υ_{max} 3417, 2928, 2685, 1665, 1614, 1516, 1464, 1385, 1222, 1176, 1058. UV (MeOH) λ_{max} (log ε) 215.0 (4.60) nm. ^1H and ^{13}C NMR data, see Table 2; positive ESIMS: *m/z* 496 $[M + H]^+$; HRESIMS: *m/z* 496.1965 $[M + H]^+$ (calcd for $C_{27}H_{30}O_8N$, 496.1966).

Caputmedusin E (5): yellow oil. IR (KBr) υ_{max} 3424, 2960, 2930, 1692, 1657, 1610, 1464, 1386, 1349, 1273, 1218, 1058 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (4.61) nm. ^1H and ^{13}C NMR data, see Table 2; positive ESIMS: *m/z* 418 $[M + H]^+$; HRESIMS: *m/z* 418.1871 $[M + H]^+$ (calcd for $C_{22}H_{28}O_7N$, 418.1860).

Caputmedusin F (6): yellow powder. IR (KBr) υ_{max} 3410, 3242, 3176, 3145, 3031, 2985, 2953, 2936, 2721, 2668, 2563, 1714, 1684, 1649, 1604, 1464, 1442, 1401, 1384, 1352, 1320, 1280, 1216, 1178, 1062 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (4.74) nm. ^1H and ^{13}C NMR data, see Table 3; negative ESIMS: *m/z* 430 $[M - H]^-$; HRESIMS: *m/z* 430.1861 $[M - H]^-$ (calcd for $C_{23}H_{28}O_7N$, 430.1871).

Caputmedusin G (7): yellow oil. IR (KBr) υ_{max} 3411, 3269, 2976, 2932, 2652, 2377, 1709, 1657, 1636, 1606, 1465, 1422, 1388, 1347, 1271, 1218, 1167, 1058 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (4.69) nm. ^1H and ^{13}C NMR data, see Table 3; negative ESIMS: *m/z* 376 $[M - H]^-$; HRESIMS: *m/z* 376.1402 $[M - H]^-$ (calcd for $C_{19}H_{22}O_7N$, 376.1402).

Caputmedusin H (8): yellow oil. IR (KBr) υ_{max} 3326, 2985, 2920, 2890, 2721, 2674, 2566, 2475, 1709, 1692, 1626, 1603, 1501, 1462, 1389, 1348, 1318, 1300, 1256, 1205, 1168, 1065 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (4.63) nm. ^1H and ^{13}C NMR data, see

Table 3
^1H and ^{13}C NMR spectroscopic data of **6–8** (δ in ppm).

No.	6		7		8	
	δ_C[a]	δ_H[b] (*J* in Hz)	δ_C[c]	δ_H[d] (*J* in Hz)	δ_C[c]	δ_H[d] (*J* in Hz)
1	169.0		171.5		171.5	
3	48.1	4.33 s	49.3	4.32 s	49.3	4.31 s
3a	132.7		131.9		131.9	
4	151.1		151.6		151.6	
5	119.7		121.3		121.3	
6	157.2		158.1		158.1	
7	101.9	6.82 s	101.9	6.73 s	101.9	6.74 s
7a	120.2		120.5		120.5	
8	23.3	3.47 d (7.2)	23.6	3.41 d (7.3)	23.6	3.39 d (7.3)
9	124.1	5.35 t (7.2)	124.2	5.30 t (7.3)	124.6	5.27 t (7.3)
10	134.5		134.5		134.2	
11	39.0	2.09 t (7.6)	36.0	2.25 t (8.1)	36.0	2.24 t (7.8)
12	27.9	2.28 m	34.1	2.35 m	33.9	2.38 t (7.8)
13	142.6	6.74 br t (7.1)	177.8		175.8	
14	128.3		16.2	1.81 s	16.1	1.79 s
15	169.1				52.0	3.55 s
16	16.2	1.82 s				
17	12.4	1.77 s				
1′	42.1	3.58 t (6.9)	43.1	3.63 t (6.3)	43.0	3.62 t (7.1)
2′	24.4	1.94 m	24.9	1.97 m	24.8	1.96 m
3′	31.5	2.36 t (7.3)	32.3	2.35 m	32.1	2.34 t (9.3)
4′	173.8		177.0		176.7	
5′	51.5	3.57 s				

[a] Measured in acetone-d_6 at 125 MHz.
[b] Measured in acetone-d_6 at 500 MHz.
[c] Measured in methanol-d_4 at 125 MHz.
[d] Measured in methanol-d_4 at 500 MHz.

Table 4
^{1}H and ^{13}C NMR spectroscopic data of **9–11** (δ in ppm).

No.	9		10		11	
	δ_C[a]	δ_H[b] (J in Hz)	δ_C[c]	δ_H[d] (J in Hz)	δ_C[c]	δ_H[d] (J in Hz)
1	171.5		170.9		170.7	
3	49.2	4.30 s	49.0	4.34 s	49.0	4.35 s
3a	131.8		123.1		123.2	
4	151.6		149.9		150.1	
5	121.3		118.1		117.7	
6	158.1		162.6		162.7	
7	101.8	6.73 s	96.0	6.62 s	96.2	6.66 s
7a	120.5		134.9		135.2	
8	23.6	3.41 d (7.2)	29.1	3.28 dd (16.0, 9.7)	29.5	3.35 dd (16.5, 9.7)
				3.18 dd (16.0, 7.6)		3.00 dd (16.5, 7.7)
9	124.3	5.30 t (7.2)	89.7	4.91 overlapped	88.6	4.98 dd (9.7, 7.7)
10	134.5		88.3		88.8	
11	36.0	2.25 t (8.5)	31.8	2.54 ddd (12.9, 10.5, 4.9)	29.0	2.26 ddd (13.3, 8.7, 7.0)
				2.14 ddd (12.9, 10.5, 7.5)		2.04 ddd (13.3, 9.9, 8.0)
12	34.0	2.35 t (8.5)	30.2	2.77 ddd (18.2, 10.5, 7.5)	30.0	2.65 overlapped
				2.64 ddd (18.2, 10.5, 4.9)		2.63 overlapped
13	177.5		179.6		179.3	
14	16.2	1.81 s	22.9	1.47 s	22.5	1.46 s
15						
1′	42.9	3.62 t (6.9)	43.2	3.63 t (7.0)	43.2	3.64 t (7.0)
2′	24.7	1.98 m	25.1	1.97 m	25.1	1.97 m
3′	32.0	2.38 t (7.3)	33.0	2.32 t (7.4)	33.1	2.32 overlapped
4′	175.2		177.9		178.0	
5′	52.1	3.58 s				

[a] Measured in methanol-d_4 at 150 MHz.
[b] Measured in methanol-d_4 at 600 MHz.
[c] Measured in methanol-d_4 at 125 MHz.
[d] Measured in methanol-d_4 at 500 MHz.

Table 3; positive ESIMS: m/z 414 $[M + Na]^+$; HRESIMS: m/z 414.1525 $[M + Na]^+$ (calcd for $C_{20}H_{25}O_7NNa$, 414.1523).

Caputmedusin I (9): yellow oil. IR (KBr) υ_{max} 3439, 3094, 2988, 2926, 2759, 2666, 2592, 1714, 1650, 1603, 1464, 1412, 1388, 1349, 1213, 1177, 1067 cm^{-1}. UV (MeOH) λ_{max} (log ε) 214.5 (4.53) nm. ^{1}H and ^{13}C NMR data, see Table 4; positive ESIMS: m/z 392 $[M + H]^+$; HRESIMS: m/z 392.1713 $[M + H]^+$ (calcd for $C_{20}H_{26}O_7N$, 392.1704).

Caputmedusin *J* (10): brown powder, $[\alpha]_D^{20}$ − 123.3° (c 0.09, MeOH). IR (KBr) υ_{max} 3405, 3243, 2974, 2935, 2676, 1762, 1659, 1611, 1497, 1458, 1422, 1384, 1353, 1295, 1274, 1245, 1213, 1170, 1074 cm^{-1}. UV (MeOH) λ_{max} (log ε) 215.4 (4.50) nm. ^{1}H and ^{13}C NMR data, see Table 4; positive ESIMS: m/z 398 $[M + Na]^+$; HRESIMS: m/z 398.1207 $[M + Na]^+$ (calcd for $C_{19}H_{21}O_7NNa$, 398.1210).

Caputmedusin K (11): brown powder, $[\alpha]_D^{20}$ + 65.3° (c 0.08, MeOH). IR (KBr) υ_{max} 3441, 2928, 2857, 2680, 1761, 1737, 1654, 1613, 1497, 1458, 1422, 1384, 1352, 1299, 1270, 1215, 1173, 1129, 1073 cm^{-1}. UV (MeOH) λ_{max} (log ε) 215.6 (4.33) nm. ^{1}H and ^{13}C NMR data, see Table 4; positive ESIMS: m/z 398 $[M + Na]^+$; HRESIMS: m/z 398.1207 $[M + Na]^+$ (calcd for $C_{19}H_{21}O_7NNa$, 398.1210).

3.4. α-Glucosidase inhibitory assay

The enzymatic inhibition assays were adapted from previously reported method [13]. A mixture containing 25 μL of different compounds (final concentrations of 0.78, 1.56, 3.13, 6.25, 12.50, 25.00, 50.00 μM), 25 μL of α-glucosidase (0.2 U/mL, from *Saccharomyces cerevisiae*, Sigma), and 175 μL of phosphate buffer (100 mM, pH 6.8) was pre-incubated for 10 min at room temperature. Then the reaction was started by the addition of 25 μL of 25 mM *p*-nitrophenyl-α-D-glucopyranoside (Sigma) and incubated for 15 min at 37 °C. The assay was conducted in a 96-well plate, and the absorbance was determined at 405 nm using a Spark™ 10 M microplate reader (Tecan). The control was prepared by adding phosphate buffer instead of the compound. The blank was prepared by adding phosphate buffer instead of α-glucosidase using the same method. The α-glucosidase inhibition rate (%) = $[(OD_{control} - OD_{control\ blank}) - (OD_{sample} - OD_{sample\ blank})] / (OD_{control} - OD_{control\ blank}) \times 100\%$. IC_{50} values were calculated through non-linear regression using GraphPad Prism5 Software. Acarbose was utilized as the positive control with an IC_{50} value of 793.79 ± 0.41 μM.

4. Conclusion

In conclusion, caputmedusins A (**1**) and B (**2**), two novel isoindolinone derivatives featuring the first dimeric isoindolinone-containing meromonoterpenoids and caputmedusins C–K (**3–11**), nine new isoindolinone-containing meroterpenoids were isolated from the fermentation broth of *Hericium caput-medusae*. Their structures were elucidated by in-depth analyses of spectroscopic data. Compounds **1–3** showed moderate inhibitory activity against α-glucosidase with the IC_{50} values of 39.2, 36.2, 40.8 μM, respectively. The isoindolinone scaffold, which is a pharmacophore of drug candidates, plays a vital role in developing new drugs. However, the biosynthetic way of this isoindolinone scaffold remains ambiguous. Isoindolinone-containing meroterpenoids, a subgroup of isoindolinone derivatives, stand out from the galaxy of natural occurrences in this group. Up to date, unravelling bioactive chemical entities from *Hericium caput-medusae* have been overwhelmingly done on the fruiting bodies. However, collecting wild fruiting bodies is always limited on the collecting season, the rarity of appropriate terrain and amounts. Therefore, the fermentation broth of *Hericium caput-medusae* emerges as an alternative method to obtain more various bioactive metabolites.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2017.08.017.

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