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Isoindolone-Containing Meroperpenoids from the Endophytic Fungus *Emericella nidulans* HDN12-249

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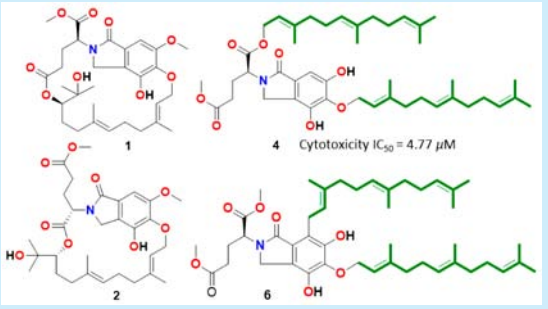
Supporting Information

ABSTRACT: Six isoindolone containing meroterpenoids, emericellolides A−C (**1**−**3**) and emeriphenolicins E−G (**4**−**6**), were isolated from a plant endophytic fungus *Emericella nidulans* HDN12-249. Emericellolides A−C (**1**−**3**) feature the unprecedented macrolide skeleton composed of an unusual L-glutamate fragment, an isoindolone unit, and a sesquiterpene moiety, while structures of emeriphenolicins E−G (**4**−**6**) with two farnesyl groups attached to one isoindolone unit are rare in isoindolone-derived meroterpenoids. These structures including the absolute configurations were established on the basis of MS, NMR, $Mo_2(AcO)_4$-induced ECD, Marfey's method, and chemical conversion. Compound **4** exhibited cytotoxicity against HeLa cells with IC_{50} value of 4.77 μM.



Meroterpenoids are natural products derived from hybrid biosynthetic origins of the isoprenoid pathway in combination with other biosynthetic pathways, such as polyketide or shikimate pathways.[1] Prenylated isoindolone alkaloids represent a family of meroterpenoids possessing an isoindolone unit and a terpene moiety. These natural products distribute broadly in microbial origins, such as the genera of *Aspergillus*,[2,3] *Emericella*,[4] *Hericium*,[5−8] *Stachybotrys*,[9−21] *Alternaria*,[22,23] etc. and demonstrate a wide range of bioactivities such as inhibition activity against α-glucosidase (erinacerins,[6] isohericerins[7]), cytotoxicity (hericerin A[8]), and plasminogen modulators (SMTPs[10−14,16−18]), and antiviral (stachyflin,[15] chartarutines,[20] stachybocins[24]) and antihyperlipidemic activities (chartarlactams[21]). Among them, over 75 cases have been reported to contain both isoindolone and farnesyl moieties (Figure S1). Generally, each molecule of these isoindolone merotepenoids possesses only one farnesyl unit, except for a few dimeric skeletons.

In the course of our ongoing search for novel secondary metabolites from plant endophytic fungi, six isoindolone-containing meroterpenoids were obtained from the culture of *Emericella nidulans* (anamorph *Aspergillus nidulans*) HDN12-249, which was isolated from the leaves of salina plant *Tamarix chinensis* Lour collected from Laizhou Bay (Figure 1). Emericellolides A−C (**1**−**3**) feature the unprecedented macrolide skeleton composing of an unusual L-glutamate fragment, an isoindolone unit, and a sesquiterpene moiety, and emeriphenolicins E−G (**4**−**6**) are isoindolone-containing meroterpenoids with two farnesyl units. Emeriphenolicin E (**4**) showed the best cytotoxicity against Hela cells with an IC_{50} value of 4.77 μM. Herein, the isolation and structural elucidation, as well as their biological properties, are described in detail.

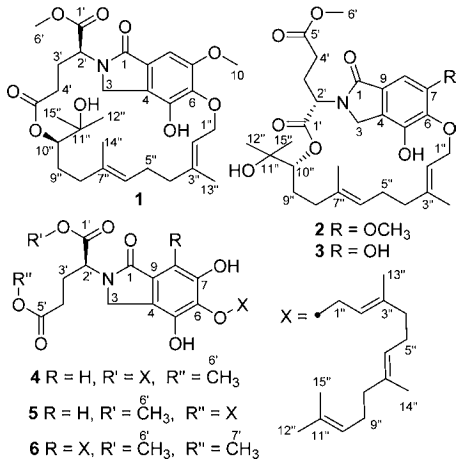


Figure 1. Structures of compounds **1**−**6**.

The fungal strain *E. nidulans* HDN12-249 was fermented (60 L) under shaking conditions at 28 °C for 9 days. The EtOAc extract (40 g) of the fermentation was fractionated by silica gel vacuum liquid chromatography (VLC), Sephadex LH-20 column chromatography, ODS MPLC, and finally HPLC to yield compounds **1** (10.0 mg), **2** (16.0 mg), **3** (9.0 mg), **4** (12.0 mg), **5** (3.0 mg), and **6** (14.0 mg).

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Compound **1** was isolated as a colorless oil with the molecular formula $C_{30}H_{41}O_9N$ according to the protonated HRESIMS peak at m/z 560.2855. The 1D NMR data (Table 1)

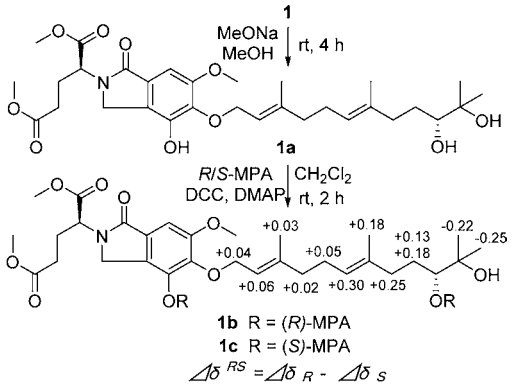
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 1 and 2 in DMSO-d_6 (δ ppm)

	1		2	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	168.4, C		168.1, C	
2				
3a	43.6, CH_2	4.24, d (16.8)	44.2, CH_2	4.58, d (16.9)
3b		4.00, d (16.8)		4.38, d (16.9)
4	121.1, C		121.6, C	
5	147.1, C		146.3, C	
6	135.8, C		135.9, C	
7	154.3, C		154.2, C	
8	97.0, CH	6.77, s	96.8, CH	6.73, s
9	126.2, C		126.1, C	
10	55.9, CH_3	3.84, s	55.8, CH_3	3.81, s
1′	170.8, C		169.2, C	
2′	52.5, CH	4.86, t (7.7)	54.4, CH	4.80, t (8.0)
3′	23.9, CH_2	2.17, m	24.0, CH_2	2.20, m
4′a	29.9, CH_2	2.38, m	29.6, CH_2	2.41, m
4′b		1.63, m		
5′	171.9, C		172.3, C	
6′	52.4, CH_3	3.66, s	51.5, CH_3	3.59, s
1″a	66.5, CH_2	4.83, dd (9.5, 11.7)	66.5, CH_2	5.07, dd (10.0, 11.8)
1″b		4.43, dd (5.4, 11.7)		4.57, dd (5.8, 11.8)
2″	118.9, CH	5.32, dd (5.4, 9.5)	120.1, CH	5.27, dd (5.8, 10.0)
3″	143.6, C		141.7, C	
4″a	39.7, CH_2	1.82, m	37.8, CH_2	1.94, m
4″b		1.58, m		1.78, m
5″a	26.3, CH_2	1.56, m	24.1, CH_2	1.96, m
5″b				1.86, m
6″	122.6, CH	4.82, t (6.9)	122.3, CH	4.11, t (6.5)
7″	134.5, C		134.1, C	
8″a	36.7, CH_2	1.95, t (12.1)	35.8, CH_2	1.33, t (12.0)
8″b		1.69, t (12.1)		1.14, t (12.0)
9″a	25.1, CH_2	1.66, m	28.4, CH_2	1.76, m
9″b		1.23, m		1.23, m
10″	80.4, CH	4.62, d (10.1)	80.1, CH	4.64, dd (1.2, 10.5)
11″	70.4, C		70.4, C	
12″	26.3, CH_3	1.00, s	26.2, CH_3	1.06, s
13″	15.3, CH_3	1.27, s	15.7, CH_3	1.44, s
14″	14.9, CH_3	1.38, s	15.7, CH_3	1.42, s
15″	24.8, CH_3	0.99, s	25.5, CH_3	1.07, s
5-OH		9.47, s		9.49, s

of **1** revealed the presence of 11 nonprotonated carbons including two ester carbonyls (δ_C 171.9, 170.8) and one amide carbon (δ_C 168.4), five methines with three sp^2 hybrids, eight methylenes, and six methyls. The chemical shifts of C-1−C-10 indicated the presence of an isoindolone moiety,[4] which was further confirmed by the HMBC correlations (Figure 2). The COSY correlations (H-2′/H-3′/H-4′) and the HMBC correlations from H-2′ and H-6′ to C-1′ and from H-3′ and H-4′ to C-5′ suggested a methylated glutamic acid residue, which was extended to the isoindolone unit according to the HMBC correlations from H-2′ to C-1 and C-3. The farnesyl group was deduced by the COSY (H-1″/H-2″, H-4″/H-5″/H-6″, and H-8″/H-9″/H-10″), and the HMBC correlations were deduced from H-13″ to C-2″, C-3″, and C-4″, from H-14″ to C-6″, C-7″, and C-8″, and from H-12″ and H-15″ to C-10″ and C-11″. The HMBC correlations between H-1″ and C-6 and between H-10″ and C-5′ connected the three units by forming an ether and an ester bonds, which lead to the establishment of the planar structure of **1**. The *E*-geometry of the double bonds $\Delta^{2'',3''}$ and $\Delta^{6'',7''}$ was determined by the NOESY correlations (H-1″/H-13″ and H-6″/H-8″).

The absolute configuration of C-2′ was assigned as *S* (L-glutamic acid) by the HPLC analysis of the FDAA derivative according to Marfey's method following the Jones oxidation and acid hydrolysis of **1** (Figure S3, SI).[24] To determine the absolute configuration of C-10″, compound **1** was methanolyzed to **1a**, whose absolute configuration was deduced as *R* by the modified Mosher's method (Scheme 1).[25] In addition, the 10″*R* configuration of **1a** was further confirmed by the obvious negative Cotton effects at 317 nm in the $Mo_2(AcO)_4$-induced ECD experiment (Figure 3).[20]

Scheme 1. Methanolysis of 1 and $\Delta\delta^{RS}$ Values for the MPA Esters



Compounds **2** and **3** were obtained with molecular formulas $C_{30}H_{41}O_9N$ and $C_{29}H_{39}O_9N$, respectively. The 1D NMR data (Table 1) of **2** are similar to those of **1** except for the chemical

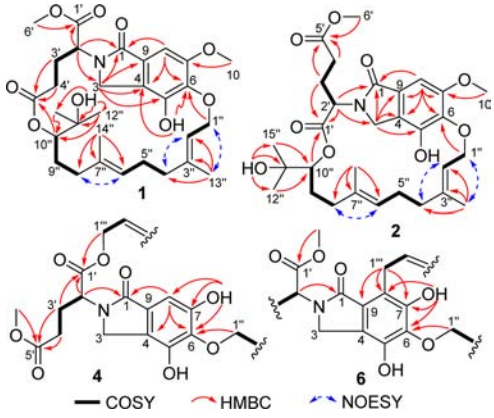


Figure 2. Key COSY, HMBC, and NOESY correlations of **1**, **2**, **4**, and **6**.

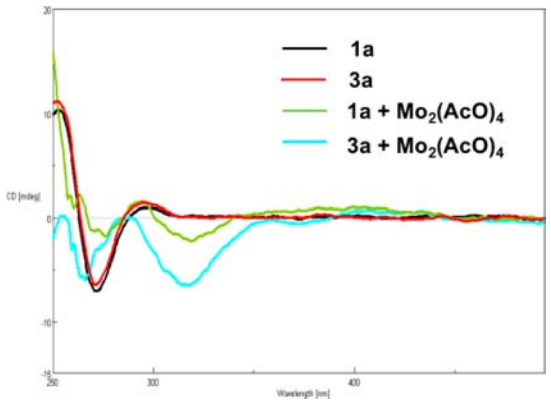


Figure 3. ECD spectra of **1a** and **3a** in DMSO solution of $[Mo_2(AcO)_4]$.

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shifts of CH_3-6′ (**1**: δ_H/δ_C 3.66, 52.4 vs **2**: δ_H/δ_C 3.59, 51.5). Further analysis of the 2D NMR correlations of **2** indicated that the methoxy group was located at C-5′ while the glutamic acid residue linked to the farnesyl group by the carboxylic acid at C-1′ instead of C-5′ as in **1**. The planar structure of **3** was determined to be almost identical to **2** with the replacement of the methoxy group at C-7 by a hydroxy. Similarly, the absolute configurations of compounds **2** and **3** were deduced to be the same as **1** by chemical conversion (**2a** and **3a** were derived from **2** and **3** by methanolysis, respectively), Marfey's methods (Figure S3), and $Mo_2(AcO)_4$-induced ECD measurement (Figure 3).

Compounds **4** and **5** were obtained as colorless oils with the same molecular formula as $C_{44}H_{63}O_8N$. Both of them were deduced to be composed of one isoindolone moiety, one glutamic acid residue, and two farnesyl groups by comparison of the NMR data (Table 2) with those of compound **1** and confirmed by the COSY and HMBC correlations. The isoindolone and glutamic acid units were linked in the same way to **1**. The two farnesyls were located on C-6 hydroxyl group and C-1′ carboxyl group, respectively, in **4** and the C-6 hydroxyl group and C-5′ carboxyl group in **5**, respectively, evidenced by the HMBC correlations from H-1″ to C-6 and from H-1‴ to C-1′ (in **4**) or C-5′ (in **5**).

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 4 and 6 in DMSO-d_6 (δ ppm)

	4		6	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	168.4, C		169.0, C	
2				
3a	44.4, CH_2	4.21, d (16.4)	43.7, CH_2	4.16, s
3b		4.16, d (16.4)		
4	120.0, C		120.3, C	
5	146.0, C		143.2, C	
6	137.3, C		137.3, C	
7	151.8, C		149.0, C	
8	101.2, C	6.68, s	118.3, C	
9	126.4, C		122.9, C	
1′	170.3, C		171.1, C	
2′	53.0, CH	4.81, dd (5.8, 10.6)	52.7, CH	4.85, dd (4.8, 10.6)
3′a	24.3, CH_2	2.24, m	24.3, CH_2	2.26, m
3′b		2.07, m		2.08, m
4′a	29.9, CH_3	2.29, t (6.5)	29.9, CH_3	2.31, t (6.4)
5′	172.4, C		172.4, C	
6′	51.3, CH_3	3.51, s	52.2, CH_3	3.65, s
7′			51.3, CH_3	3.51, s
1″	68.1, CH_2	4.53, d (6.8)	68.3, CH_2	4.53, d (6.8)
2″	120.6, CH	5.52, t (6.8)	120.3, CH	5.54, t (6.8)
3″	140.0, C		140.3, C	
4″	39.1, CH_2	1.96, t (7.0)	39.2, CH_2	1.92, t (8.0)
5″	25.9, CH_2	1.91, m	25.9, CH_2	1.98, m
6″	123.7, CH	5.07, t (6.6)	123.7, CH	5.06, t (6.6)
7″	134.7, C		134.5, C	
8″	39.2, CH_2	2.01, t (7.6)	39.4, CH_2	1.86, t (6.8)
9″	26.2, CH_2	1.99, m	26.2, CH_2	1.98, m
10″	124.1, CH	5.04, t (7.0)	124.1, CH	5.05, t (6.8)
11″	130.6, C		130.6, C	
12″	25.5, CH_3	1.62, s	25.5, CH_3	1.62, s
13″	16.1, CH_3	1.58, s	16.1, CH_3	1.53, s
14″	15.7, CH_3	1.54, s	15.7, CH_3	1.52, s
15″	17.5, CH_3	1.54, s	17.5, CH_3	1.53, s
1‴a	61.6, CH_2	4.58, d (7.0)	22.3, CH_2	3.70, dd (6.5, 16.5)
1‴b				3.67, dd (6.5, 16.5)
2‴	118.0, CH	5.25, t (7.0)	123.6, CH	5.21, t (6.5)
3‴	142.2, C		133.1, C	
4‴	38.9, CH_2	1.96, t (7.0)	39.2, CH_2	1.92, t (8.0)
5‴	26.1, CH_2	1.91, m	26.2, CH_2	1.98, m
6‴	123.5, CH	5.06, t (6.6)	123.9, CH	5.03, t (6.8)
7‴	134.5, C		134.2, C	
8‴	39.2, CH_2	2.01, t (7.6)	39.4, CH_2	1.86, t (6.8)
9‴	26.1, CH_2	1.98, m	26.2, CH_2	1.98, m
10‴	124.1, CH	5.04, t (7.0)	124.1, CH	5.02, t (7.0)
11‴	130.6, C		130.5, C	
12‴	25.5, CH_3	1.62, s	25.4, CH_3	1.61, s
13‴	16.2, CH_3	1.62, s	16.0, CH_3	1.72, s
14‴	15.7, CH_3	1.54, s	15.7, CH_3	1.50, s
15‴	17.5, CH_3	1.54, s	17.5, CH_3	1.54, s
5-OH		9.37, s		9.24, s
7-OH		9.60, s		8.51, s

The molecular formula of **6** was suggested as $C_{45}H_{65}O_8N$ by the protonated HRESIMS ion at m/z 748.4784. Unlike compounds **4** and **5**, both of the two farnesyls were located on the isoindolone moiety at 6-O and C-8, evidenced by the HMBC correlations from H-1″ to C-6 and from H-1‴ to C-7, C-8 and C-9 (Figure 2), while the two carboxylic acids of glutamic acid residue were formed to esters with two methyl groups supported by the HMBC correlations (Figure 2). The absolute configurations of compounds **4**–**6** were also determined as 2′*S* by Marfey's methods (Figure S3).

The biogenetic pathway of isoindolone structures has been proposed to begin from the key intermediate asperugin A or B.[26] Similarly, for hybrids with polyketide, L-glutamate, and farnesyl units, compounds **1**–**6** are proposed to be formed by further post modification such as methylation, oxidation, and intramolecular esterification (Scheme S1).

To exclude that compounds **1**–**6** are artifacts formed in the process of extraction and purification using EtOAc and MeOH, the fresh culture of *E. nidulans* HDN12-249 was lyophilized and extracted with MeCN. As a result, all of the compounds can be detected by LC–UV–MS analysis (Figure S4).

Compounds **1**–**6** were screened for cytotoxic activity in three human cancer cell lines, including HeLa, A549, and HCT-116 (adriamycin as positive control).[27,28] Compound **4** displayed selective activity with IC_{50} values of 4.77, 12.04, and 33.05 μM, respectively, while others were inactive (IC_{50} > 50 μM).

In summary, six new isoindolone-derived meraterpenoids were discovered from the culture of the endophytic fungus *Emericella nidulans* HDN12-249. Emericellolides A–C (**1**–**3**) feature the unprecedented macrolide skeleton, while emeriphenolicins E–G (**4**–**6**) contain two farnesyl groups in diverse positions (6-O, C-8, 1′-O, and 5′-O) via different linkages (ester, ether, and C–C bonds). Compounds **4**–**6** are the first instances with two farnesyl substituents attached to one single isoindolone subunit, indicating a group of unique enzymes with particular biochemical reaction mechanisms preserved in this fungus.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02297.

Experimental details and procedures, HRESIMS, NMR spectra of compounds **1**–**6** (PDF)

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Notes

The authors declare no competing financial interest.

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