SUPPLEMENTARY MATERIAL

A new lumazine peptide penilumamide E from the fungus *Aspergillus terreus*

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Abstract

A new rare lumazine peptide, penilumamide E (**1**), together with thirteen known compounds were isolated from the fungus *Aspergillus terreus*. Their structures were identified by spectroscopic techniques. The relative configuration of **1** was confirmed by single-crystal X-ray diffraction analysis. Compound **10** exhibited antimalarial activity against *Plasmodium falciparum* with IC₅₀ values of 2.83 µg/ml. Compounds **4** and **6** showed weak cytotoxicity against cholangiocarcinoma (CCA) cell lines. In addition, **4** and **11** exhibited weak cytotoxicity against Human hepatoma cell line.

Keywords: *Aspergillus terreus*, lumazine peptide, penilumamide E, *epi*-azonalenin A, butyrolactone, antimalarial

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Content

Table S1. ^1H and ^{13}C NMR of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at (400 MHz)	3
Table S2. Crystallographic data for compound 1	4
Table S3. Selected bond lengths ($\text{\AA}$) and angles (deg) for compound 1	5
Figure S1. X-ray crystallographic structure of compound 1 .	5
Figure S2. Mass spectrum of 1 .	6
Figure S3. IR spectrum of 1 .	6
Figure S4. ^1H -NMR spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 400 MHz.	7
Figure S4a. Expansion of ^1H -NMR spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	7
Figure S5. ^{13}C -NMR spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 100 MHz.	8
Figure S6. DEPT spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	8
Figure S7. HMQC spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	9
Figure S8. HMBC spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	9
Figure S8a. expansion of HMBC spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	10
Figure S8b. expansion of HMBC spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	10
Figure S9. COSY spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	11
Figure S10. NOESY spectrum of 1 recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$.	12
X-ray crystallographic analysis of 1	13
Biological activity procedures	14
References	15

Table S1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data (δ , ppm) for compound **1** in $\text{CDCl}_3/\text{CD}_3\text{OD}$

position	δ_{H}	δ_{C}	HMBC	NOESY
2		149.8 s ^b		
3	11.87, brs ^a			
4		160.4 s		
4a		125.7 s		
5				
6		139.6 s		
7	9.37, s	148.1 d	4a, 6, 8a	
8				
8a		150.7 s		
9	3.65, s	28.9 q	2, 8a	
10		163.2 s		
1'-NH	9.08, d (8.0)		10	
2'	4.63, d (3.6)	60.8 d	10, 3', 4', 5'	5'
3'		169.0 s		
4'	4.54, m	67.2 d	3', 5'	5'
5'	1.31, d (6.0)	19.6 q	2', 4'	2', 4'
1''-NH	11.55, s		3', 7''	
2''		140.1 s		
3''		115.7 s		
4''	7.91, d (7.2)	130.7 d	2'', 6'', 8''	5''
5''	7.05, t (7.2)	123.1 d	3'', 7''	4'', 6''
6''	7.47, t (7.2)	134.3 d	2'', 4''	5'', 6''
7''	8.59, d (8.4)	120.4 d	3'', 5''	6''
8''		168.2 s		
9''	3.66, s	52.0 q	8''	

^aFigures in parentheses are coupling constants in Hz.^bMultiplicities were deduced from DEPT and/or HSQC experiments.

Table S2. Crystallographic data for compound **1**

Compound	1
Formula	C ₂₀ H ₂₀ N ₆ O ₇
Molecular weight	456.42
<i>T</i> (K)	273(2)
Crystal system	monoclinic
Space group	<i>P</i> 2 ₁
<i>a</i> (Å)	9.5671(4)
<i>b</i> (Å)	6.7995(3)
<i>c</i> (Å)	16.5142(6)
α (deg)	90.00
β (deg)	103.8450(10)
γ (deg)	90.00
<i>V</i> (Å ³)	1043.06(7)
<i>Z</i>	2
ρ_{calcd} (g cm ⁻³)	1.453
μ (Mo <i>K</i> α) (mm ⁻¹)	0.113
Data collected	17586
Unique data	4117
R_I^a / wR_2^b [<i>I</i> > 2 σ (<i>I</i>)]	0.0394/ 0.0943
R_I^a / wR_2^b [all data]	0.0553/ 0.1024
GOF	1.063
Max/min electron density (e Å ⁻³)	0.20/-0.15

$$^a R = \sum ||F_o| - |F_c|| / \sum |F_o|, \quad ^b R_w = \{ \sum [w(|F_o| - |F_c|)]^2 / \sum [w|F_o|^2] \}^{1/2}$$

Table S3. Selected bond lengths (Å) and angles (deg) for compound **1**

Compound 1			
N1–C2	1.382(4)	Co1–N6	2.141(5)
Co1–N3 ⁱ	2.116(4)	Co1–N1 ⁱⁱⁱ	2.193(4)
Co1–N6 ⁱⁱ	2.141(5)	Co1–N1	2.193(4)
N3–Co1–N3 ⁱ	78.3(3)	N6 ⁱⁱ –Co1–N1 ⁱⁱⁱ	88.10(8)
N3–Co1–N6 ⁱⁱ	170.35(19)	N6–Co1–N1 ⁱⁱⁱ	88.10(8)
N3 ⁱ –Co1–N6 ⁱⁱ	92.10(19)	N3–Co1–N1	92.24(9)
N3–Co1–N6	92.10(19)	N3 ⁱ –Co1–N1	92.24(9)
N3 ⁱ –Co1–N6	170.35(19)	N6 ⁱⁱ –Co1–N1	88.10(8)
N6 ⁱⁱ –Co1–N6	97.5(3)	N6–Co1–N1	88.10(8)
N3–Co1–N1 ⁱⁱⁱ	92.24(9)	N1 ⁱⁱⁱ –Co1–N1	174.2(2)
N3 ⁱ –Co1–N1 ⁱⁱⁱ	92.24(9)	N6–N7–N6 ^{iv}	176.2(8)
Co1–N3–Co1 ⁱ	101.7(3)	N5–N4–N3	178.1(13)
N7–N6–Co1	129.3(5)

Figure S1. X-ray crystallographic structure of compound **1**.

Figure S2. Mass spectrum of **1**.

Figure S3. IR spectrum of **1**.

Figure S4. ^1H -NMR spectrum of **1** recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 400 MHz.

Figure S4a. Expansion of ^1H -NMR spectrum of **1** recorded in CDCl_3 at 400 MHz

Figure S5. ^{13}C -NMR spectrum of **1** recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 100 MHz.

Figure S6. DEPT spectrum of **1** recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 100 MHz.

Figure S7. HMQC spectrum of **1** recorded in CDCl₃/CD₃OD at 400 MHz.

Figure S8. HMBC spectrum of **1** recorded in CDCl₃/CD₃OD at 400 MHz.

Figure S8a. expansion of HMBC spectrum of **1** recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 400 MHz.

Figure S8b. expansion of HMBC spectrum of **1** recorded in $\text{CDCl}_3/\text{CD}_3\text{OD}$ at 400 MHz.

Figure S9. COSY spectrum of **1** recorded in CDCl₃/CD₃OD at 400 MHz.

Figure S10. NOESY spectrum of **1** recorded in CDCl₃/CD₃OD at 400 MHz.

1. X-ray crystallographic analysis of **1**

All reflection data were collected on a Bruker D8-Quest PHOTON-100 CMOS detector with graphite-monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$) using the APEX2 program (Bruker, 2014). Raw data frame integration was performed with SAINT (Bruker, 2014). An empirical absorption correction was applied to the data using the SADABS program (Bruker, 2014). The structure was solved by direct methods and refined by the full-matrix least-squares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms using the SHELXTL2014 software package (Sheldrick, 2008). All hydrogen atoms were placed in calculated positions and refined isotropically with a riding model. Crystallographic data (excluding structure factors)

have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 1403813. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.

2. Biological activity procedures

2.1. Antimalarial assay.

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen (Trager and Jensen 1967). Quantitative assessment of activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins and co-workers (Desjardins et al. 1979). The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [3H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (IC_{50} 0.001 μ g/ml).

2.2. Cytotoxicity assays

Cytotoxicity assays against human epidermoid carcinoma (KB), human breast adenocarcinoma (MCF7), and human small cell lung cancer (NCI-H187) were performed employing the Resazurin microplate assay (REMA) described by and co-workers (O'Brien et al. 2000). The reference substance was doxorubicin.

2.3 KKU-100 and HepG2 cells assays

The human cholangiocarcinoma (CCA) cell line (KKU-100 cells) and Human hepatoma cell line (HepG2 cells) were seeded onto a 96 well-plate at a density of 7.5×10^3 and 1.5×10^4 cells/well, respectively. After an overnight incubation, cultured media were changed to fresh media containing tested compounds. Test compounds, dissolved in DMSO and diluted with medium to various concentrations, were added into cultured cells and incubated for 24 h. Cisplatin was included for the purpose of comparison. The cytotoxicity was assessed by the sulforhodamine B (SRB) assay as previously described (Tussekorn et al. 2013). In brief, cultured cells were fixed with 10% trichloroacetic acid and stained with 0.4% SRB. The protein-bound dye was solubilized with 10 mM Tris-base solution for determination of the

absorbance at 540 nm with a microplate reader. The cytotoxicity was calculated as percent absorbance of controls. The IC₅₀ value was calculated by a non-linear curve-fitting program.

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