SUPPLEMENTARY MATERIAL

New naphthopyrones from marine-derived fungus *Aspergillus niger* 2HL-M-8 and their *in vitro* antiproliferative activity

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Abstract: A new cytotoxic dimeric naphthopyrone, aurasperone H (**1**), together with eight related known polyketides (**2-9**) was isolated from a marine-derived fungus *Aspergillus niger* 2HL-M-8. The structure of new compound **1** was elucidated on the basis of its spectroscopic data (1D, 2D NMR and CD). Compound **1** exhibited moderate inhibitory activity against the human lung adenocarcinoma A549 and the human leukemia HL-60 cell lines. Compound **5** displayed significant *in vitro* antiproliferative activity against HL-60 cell line with an IC₅₀ value of 0.8 μM.

Keywords: *Aspergillus niger*; dimeric naphthopyrones; antiproliferative activity

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Figure S1. Key HMBC correlations of compound **1**.

Figure S2. ^1H NMR spectrum of **1** (600 MHz in CDCl_3).

Figure S3. ^{13}C NMR spectrum of **1** (150 MHz in CDCl_3).

Figure S4. HSQC spectrum for compound **1** in CDCl₃.

Figure S5. HMBC spectrum for compound **1** in CDCl₃.

Figure S6. NOESY spectrum for compound **1** in CDCl₃.

Figure S7. HR-ESI-MS spectrum of **1**.

Generate Molecular Formula Parameter

Formula, min. C₃₄H₃₀O₁₁H

Formula, max.

Measured m/z 615.186

Check Valence no

Nitrogen Rule no

Filter H/C Ratio no

Estimate Carbon yes

Tolerance 5 ppm
Minimum 0
Electron Configuration both
Minimum 0

Charge 1
Maximum 0
Maximum 3

Compound **1** (0.5 mg) was dissolved in a dry solution of $[\text{Rh}_2(\text{OCOCF}_3)_4]$ complex (1.5 mg) in CDCl_3 (200 μL). The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (ca. 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-2'' secondary alcohol moiety.

Figure S8. CD spectrum of **1**.

(a) : CD spectrum of **1**;

(b) : CD spectrum after reaction with $\text{Rh}_2(\text{OCOCF}_3)_4$ of compound **1**;

(c) : CD spectrum of the Rh complex of **1** with the inherent CD spectrum subtracted.

Figure S9. The morphological changes of HL-60 cells upon different concentrations of compound **5**. (A) Negative control: 0.1% DMSO; (B) 0.78 μM of **5**; (C) 1.56 μM of **5**; (D) 3.12 μM of **5**; (E) 6.24 μM of **5**; (F) Positive control: 10 $\mu\text{g/mL}$ of paclitaxel.

Table 1. NMR data (600 MHz for ^1H and 150 MHz for ^{13}C , CDCl_3) of compound **1**.

Position	δ_{H} (J in Hz)	δ_{C}	HMBC ($\text{H} \rightarrow \text{C}$)	NOESY
2		167.6		
3	6.06, s	107.4	C-2, C-4a	2-CH ₃
4		184.4 ^c		
4a		104.8		
5		161.9		
5a		111.1		
6		158.3		
7		117.3		
8		159.8		
9	7.00, s	101.7	C-5a, C-7, C-8, C-10	H-10, 8-OCH ₃
9a		140.4 ^d		
10	7.16, s	101.3	C-4a, C-5a, C-9, C-10a	H-9
10a		153.4		
2'		168.1		
3'	6.03, s	108.6	C-1'', C-2', C-4'a	
4'		184.5 ^c		
4'a		104.6		
5'		162.9		
5'a		108.4		
6'		161.1		
7'	6.43, d ($J = 2.1$)	96.9	C-5'a, C-8', C-9'	6'-OCH ₃ , 8'-OCH ₃
8'		161.5		
9'	6.26, d ($J = 2.1$)	96.5	C-5'a, C-7', C-8', C-10'	6-OCH ₃ , 8'-OCH ₃
9'a		140.5 ^d		
10'		104.8		
10'a		150.6		
2-CH ₃	2.42, s	20.8	C-2, C-3	H-3
6-OCH ₃	3.43, s	62.2	C-6	H-9'
6'-OCH ₃	4.03, s	56.2	C-6'	H-7'

8-OCH ₃	3.80, s	55.9	C-8	H-9
8'-OCH ₃	3.66, s	55.2	C-8'	H-7', H-9'
5-OH	14.76, s		C-4a, C-5a, C-5	
5'-OH	15.21, s		C-4'a, C-5'a, C-5'	
1''	2.53, m	43.6	C-2', C-2'', C-3''	H-2'', H-3''
2''	3.96, m	64.6		H-1'', H-3''
3''	1.09, d (<i>J</i> = 6.2)	22.9	C-1'', C-2''	H-1'', H-2''
2''-OH	2.89, d (<i>J</i> = 5.3)			

^aRecorded at 600 MHz. ^bRecorded at 150 MHz. ^{c,d}Signals are interchangeable.

Table S2. NMR data of **2** in CDCl₃.

position	δ^a_{H} (J in Hz)	δ^b_{C}	HMBC (H $\rightarrow$ C)	NOESY
2		167.8		
3	6.05, d (J = 0.6)	107.6	C-4a, C-4, 2-CH ₃ , C-2	2-CH ₃
4		184.8		
4a		104.9		
5		162.2		
5a		111.6		
6		158.8		
7		117.8		
8		160.4		
9	6.97, s	101.5	C-8, C-10, C-5a, C-7	8-OCH ₃ , H-10
9a		140.9		
10	7.15, s	101.4	C-9, C-4a, C-5a, C-10a	H-9
10a		153.5		
2'		167.7		
3'	5.98, d (J = 0.6)	107.4	C-4'a, C-4', C-2', 2'-CH ₃	2'-CH ₃
4'		184.6		
4'a		104.5		
5'		162.9		
5'a		108.7		
6'		161.2		
7'	6.41, d (J = 2.1)	97.0	C-9', C-5'a, C-8'	8'-OCH ₃ , 6'-OCH ₃
8'		161.6		
9'	6.21, d (J = 2.1)	96.7	C-7', C-5'a, C-9'a, C-8'	6-OCH ₃ , 8-OCH ₃ , 8'-OCH ₃
9'a		140.7		
10'		105.3		
10'a		151.0		
2-CH ₃	2.41, s	21.0	C-3, C-2	H-3
2'-CH ₃	2.12, s	20.9	C-2', C-3'	H-3'
6-OCH ₃	3.46, s	62.2	C-6	H-9'
6'-OCH ₃	4.02, s	56.4	C-6'	H-7'
8-OCH ₃	3.78, s	56.1	C-8	H-9
8'-OCH ₃	3.62, s	55.3	C-8'	H-7', H-9'
5-OH	14.83, s			
5'-OH	15.23, s			

^aRecorded at 300 MHz. ^bRecorded at 150 MHz.

Table S3. ¹H NMR data for compounds **3-9** in CDCl₃.

proton	3^b	4^b	5^b	6^a	7^a	8^b	9^a
3	6.33, s	6.05, s	6.34, s	3.04, m	6.32, s	6.03, s	6.27, s
6	7.05, s		7.04, s		7.04, s		6.87, s
7	6.97, s		6.99, s		6.98, s		6.40, d (<i>J</i> = 2.1)
9		6.98, s		6.84, s		6.98, s	6.59, d (<i>J</i> = 2.1)
10		7.14, s		6.70, s		7.12, s	
2-CH ₃	2.48, s	2.41, s	2.48, s	1.81, s	2.48, s	2.40, s	2.49, s
6-OCH ₃		3.43, s		3.42, s		3.64, s	
8-OCH ₃	3.78, s	3.82, s	3.84, s	3.76, s	3.80, s	3.81, s	3.97, s
10-OCH ₃	3.43, s		3.39, s		3.61, s		3.92, s
5-OH	12.83, s	14.78, s	12.78, s	14.15, s	12.73, s	14.75, s	12.79, s
3'	6.00, s	2.93, m	2.95, m	5.97, s	6.33, s	6.32, s	
7'	6.43, d (<i>J</i> = 2.2)	6.36, d (<i>J</i> = 1.8)	6.38, d (<i>J</i> = 2.2)	6.41, d (<i>J</i> = 2.0)	6.20, d (<i>J</i> = 2.0)	6.26, d (<i>J</i> = 2.1)	
9'	6.19, d (<i>J</i> = 2.2)	6.13, d (<i>J</i> = 1.8)	6.14, d (<i>J</i> = 2.2)	6.21, d (<i>J</i> = 2.0)	6.44, d (<i>J</i> = 2.0)	6.42, d (<i>J</i> = 2.1)	
2'-CH ₃	2.12, s	1.48, s	1.46, s	2.12, s	2.55, s	2.53, s	
6'-OCH ₃	4.03, s	4.00, s	4.00, s	4.02, s			
8'-OCH ₃	3.62, s	3.64, s	3.64, s	3.63, s	3.61, s	3.61, s	
10'-OCH ₃					4.01, s	3.99, s	
5'-OH	15.23, s	14.53, s	14.53, s	15.22, s	13.10, s	13.11, s	

^aRecorded at 300 MHz. ^bRecorded at 600 MHz.

Table S4. ^{13}C NMR data for compounds **3-9**^a in CDCl_3 .

carbon	3	4	5	6	7	8	9
2	167.7	167.9	166.9	100.1	167.0	167.7	166.8
3	110.9	107.6	110.9	47.3	110.8	107.6	110.4
4	183.1	184.7	183.1	196.5	183.1	184.7	183.0
4a	109.6	104.9	109.7	104.3	109.5	104.8	109.0
5	156.9	162.3	156.7	164.1	156.8	162.1	156.8
5a		111.6		110.7		111.7	
6	106.2	157.8	102.2	159.5	106.4	158.5	105.1
6a	141.0		140.6		140.9		141.4
7	101.8	116.8	106.2	116.9	102.1	118.5	98.1
8	160.2	160.4	162.1	161.0	160.2	160.4	161.6
9	117.3	101.9	118.4	102.7	118.1	101.9	97.2
9a		140.3		140.7		140.8	
10	157.1	101.4	160.2	101.9	156.7	101.4	159.2
10a	108.2	153.4	106.7	153.3	108.3	153.4	106.0
10b	155.3		155.7		155.2		156.0
2'	167.0	100.4	100.4	167.6	166.8	166.7	
3'	107.6	47.1	46.8	107.2	110.5	110.4	
4'	184.7	197.8	197.4	184.6	183.2	183.2	
4'a	104.4	104.0	103.9	103.5	108.6	108.6	
5'	163.0	165.1	165.3	162.7	154.5	154.4	
5'a	108.8	108.1	108.1	105.1			
6'	161.3	160.4	160.2	161.0	109.9	110.1	
6'a					141.1	140.9	
7'	97.2	97.5	96.6	96.5	96.6	96.9	
8'	161.8	162.3	162.6	161.5	161.7	161.6	
9'	96.5	96.3	97.4	97.0	97.0	96.9	
9'a	140.8	140.3	142.7	142.7			
10'	105.2	107.6	106.7	108.6	159.7	159.6	
10'a	151.0	153.4	151.3	150.8	105.2	105.2	
10'b					156.0	155.9	
2-CH ₃	20.8	21.0	20.8	28.8	20.8	20.9	20.7
2'-CH ₃	20.9	29.6	29.3	20.7	20.8	20.7	
6-OCH ₃		62.0		61.9		62.4	
8-OCH ₃	56.2	56.1	56.2	55.9	56.3	56.3	55.6
6'-OCH ₃	56.4	56.4	56.4	56.2			
8'-OCH ₃	55.4	55.3	55.4	55.2	61.7	55.4	
10-OCH ₃	61.4		61.3		55.4		56.1
10'-OCH ₃					56.2	56.2	

^aRecorded at 150MHz.