Supplementary Materials for

Psychrophilin E, A New Cyclotriptide, from Co-Fermentation of Two Marine Alga-Derived Fungi of the Genus *Aspergillus*

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Chemical investigation of the mycelial extract of a mixed culture of two marine alga-derived fungal strains of the genus *Aspergillus* has afforded one new cyclotriptide, psychrophilin E (**1**), the recently reported oxepin-containing alkaloids, protuboxepin A (**2**) and oxepinamide E (**3**), together with three other polyketides derivatives (**4–6**). The chemical structure, relative and absolute configurations of psychrophilin E (**1**) were unambiguously established based on HRMS, 1D, 2D NMR, and chiral-phase HPLC analysis of its hydrolysate. All the isolated compounds were assessed for their cytotoxic activity against four different human cancer cell lines and some of them revealed selective antiproliferative activities.

Keywords: *Aspergillus*, co-fermentation, marine-derived fungi, antiproliferative

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Table S1. 1D and 2D NMR data for psychrophilin E (**1**)

#	δ_{H}^a (mult., J in Hz)	δ_{C}^a (mult.)	COSY ^{a,c}	HMBC ^{a,c}	ROESY ^b
1		172.4, C			
2	5.08 (ddd, 1H, 5.5, 7.9, 13.1)	51.0, CH	2-NH, 3a/b ^e	1, 3, 24	3a/3b ^e , 6, 23a/b, 2-NH
3	b: 3.35 (dd, 1H, 5.4, 12.2)	29.3, CH ₂	2 ^e , 3b	1, 2, 4, 5 ^e , 11	2, 6, 25 ^e , 2-NH ^e
	a: 2.89 (t, 1H, 12.2)		2, 3a	1, 2, 4, 5, 11	2 ^e , 11, 25 ^e , 2-NH
4		116.4, C			
5		129.6, C			
6	7.67 (d, 1H, 7.6)	118.3, CH	7	4, 8, 10	2, 3b
7	7.38 (t, 1H, 7.3)	124.5, CH	6, 8	5, 9	
8	7.43 (t, 1H, 7.6)	126.1, CH	7, 9	6, 7 ^e , 10	
9	8.62 (d, 1H, 8.1)	117.2, CH	8	5, 7, 10 ^e	
10		135.5, C			
11	6.86 (s, 1H)	125.6, CH		2 ^e , 3, 4, 5, 10, 12 ^e	3a, 18-NH
12		166.2, C			
13		128.1, C			
14	7.82 (d, 1H, 7.6)	131.7, CH	15	12, 16, 18	
15	7.38 (t, 1H, 7.3)	126.3, CH	14, 16	13, 17	

16	7.57 (t, 1H, 7.3)	132.5, CH	15, 17	14, 18	18-NH
17	7.57 (d, 1H, 7.6)	123.7, CH	16	13, 15	18-NH
18		133.4, C			
19		167.4, C			
20	4.55 (dd, 1H, 2.7, 8.3)	59.8, CH	21a/b	1 ^e , 19, 21 ^e , 22, 23	21a/21b, 23a, 18-NH
21	a: 2.02 (m, 1H)	26.3, CH ₂	20, 21/b	23 ^e	20, 23a ^e
	b: 1.69 (m, 1H)		20 ^e , 21/a	19, 20 ^e , 23 ^e	20, 23a ^e
22	a: 1.52 (m, 1H)	24.7, CH ₂	22b, 23a/b	20 ^e	23a/b ^e
	b: 1.23 (m, 1H)		22a, 23a/b	23 ^e	23a ^e /b
23	a: 3.27 (dd, 1H, 9.2, 16.7)	47.7, CH ₂	22a/b, 23b	1 ^e , 20 ^e , 21 ^e , 22	21a/b, 22a/b
	b: 2.14 (m, 1H)		22a/b, 23a	20 ^e , 21 ^e , 22	21b, 22a/b
24		170.1, C			
25	2.04 (s, 3H)	23.0, CH ₃		2 ^e , 24	3a ^e /3b ^e , 2-NH
2-NH	6.49 (brd, 1H, 7.6)		2	24	2, 3a/b ^e , 25
18-NH	8.50 (brs, 1H)			13, 17, 18 ^e , 19 ^e	11, 16, 17, 20

^aCDCl₃, 500/125 MHz. ^bCDCl₃, 600/150 MHz. ^cAssignments are based on extensive 1D and 2D NMR measurements (HMBC, HSQC, COSY). ^dNumbers refer to carbon resonances. ^eWeak signals.

RT: 19.81 - 35.10

RT: 19.81 - 34.92

RT: 19.86 - 34.24

Figure S1. HPLC-MS of 1

Figure S2. ESIMS spectrum of 1

Figure S3. UV spectrum of 1

Figure S4. IR spectrum of **1**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source

Figure S5. HRESIMS spectrum of **1**

Figure S6. ^1H NMR spectrum of **1** in CDCl_3

Figure S7. ^{13}C NMR spectrum of **1** in CDCl_3

Figure S8. DEPT spectrum of **1** in CDCl_3

Figure S9. ^1H - ^1H COSY spectrum of **1** in CDCl_3

Figure S10. HSQC spectrum of **1** in CDCl_3

Figure S11. HMBC spectrum of **1** in CDCl_3

Methylation

Acetylation

Figure S12. Proline enantioresolution by chiral GC

Figure S13. Chiral GC Analysis of derivatized D and L Proline on CP-chirasil Dex compared to that from hydrolysate

Figure S14. ROESY spectrum of **1** in CDCl₃

Figure S15. 3D models of the minimal energy conformation of (2*R*,20*S*) and (2*S*,20*S*) psychrophilin E (Psp E, **1**). The ROESY correlations, shown with arrows, illustrate that C-2 most likely has the *R* configuration. Left: 2*R*,20*S* configuration and minimal energy conformation (46.6 kcal/mol). Right: 2*S*,20*S* configuration and minimal energy conformation (65.8 kcal/mol).