SUPPLEMENTARY MATERIAL

Secondary metabolites from marine-derived *Streptomyce antibioticus* strain

H74-21

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A new secondary metabolite, (2*S*,3*R*)-*L*-Threonine, *N*-[3-(formylamino)-2-hydroxybenzoyl]-ethyl ester (streptomyceamide C, **1**), together with four known compounds **1**, 4-dimethyl-3-isopropyl-2,5-piperidinedione (**2**), cyclo-((*S*)-Pro-8-hydroxy-(*R*)-Ile (**3**), cyclo-((*S*)-Pro-(*R*)-Leu (**4**), and seco-((*S*)-Pro-(*R*)-Val) (**5**), were isolated from the EtOH extract of the fermented mycelium of the marine-derived *streptomycete* strain H74-21, which was isolated from sea sediment in a mangrove district. The structure of the new compound was established on the basis of its spectroscopic data, including 1D NMR and 2D NMR, HR-TOF-MS. Their antifungal activities against *Candida albicans* and cytotoxicities against human breast adenocarcinoma cell line MCF-7, human glioblastoma cell line SF-268 and human lung cancer cell line NCI-H460 were tested. Compounds **1** only displayed cytotoxicity against human breast adenocarcinoma cell line MCF-7 with the IC₅₀ value of 27.0 µg/mL. However, compounds **1-5** do not show antifungal activities at the test concentration of 1 mg/mL, and **2-5** have no cytotoxicities at the test concentration of 50 µg/mL.

Keywords: *Streptomycete*; secondary metabolites; dipeptide; streptomyceamide C;

piperidinedione

Compound 1

Figure S1. The IR spectrum of Compound 1

Figure S2. HR-MS of compound 1

Figure S3. The ¹H spectrum of compound **1** (300 MHz in CD₃OD)

Figure S4. The ¹³C spectrum of compound **1** (75 MHz in CD₃OD)

Figure S5. The DEPT spectrum of compound **1** (in CD_3OD)

Figure S6. The ^1H - ^1H COSY spectrum of compound **1** (in CD_3OD)

Figure S7. The HMBC spectrum of compound **1** (in CD₃OD)

Figure S8. The HMBC spectrum of compound **1** (in CD₃OD)

Figure S9. The NOE spectrum of compound **1** (300 MHz in CD₃OD)

Figure S10. The ¹H spectrum of compound **1** (600 MHz in DMSO-*d*₆)

Figure S11. The NOE spectrum of compound **1** (600 MHz in DMSO- d_6)

Figure S12. The rational or alternative configuration and their favorite conformers with the lowest energy of compound **1** in ChemDraw. a: A rational configuration for **1**, In its favorite conformer, dihedral angle between H(8)-C(8)-C(13) and H(13)-C(13)-C(8) is about 120° (Karplus equation); b: An alternative and less possible configuration for **1**, in it favorite conformer, dihedral angle between H(8)-C(8)-C(13) and H(13)-C(13)-C(8) is about 180° .

Figure S13. The observed NOE correlations of compound **1**

Figure S14. The ¹H-¹H COSY and key HMBC correlations of **1** and **2**

Table S1. ¹H (300 MHz) and ¹³C (75 MHz) NMR Data of **1** and **2** (δ in ppm *J* in Hz)

Position	1 (in CD ₃ OD)		2 (in CDCl ₃)	
	δ (H)	δ (C)	δ (H)	δ (C)
1		115.7		
2		152.6		167.4
3		128.2	3.83 (1H, d, <i>J</i> = 4.3)	69.4
4	8.30 (1H, d, <i>J</i> = 7.9)	126.4		
5	6.91 (1H, t, <i>J</i> = 7.9)	119.6		166.8
6	7.64 (1H, d, <i>J</i> = 7.9)	123.5	4.18 (1H, d, <i>J</i> = 18.0) 3.90 (1H, d, <i>J</i> = 18.0)	52.7
7		171.9	2.95 (3H, s)	33.7
8	4.71 (1H, d, <i>J</i> = 6.9)	59.7	2.30 (1H, m)	33.9
9		172.1	0.94 (3H, d, <i>J</i> = 6.9)	18.2
10	4.21 (2H, q, <i>J</i> = 7.1)	62.8	1.06 (3H, d, <i>J</i> = 7.0)	19.9
11	1.29 (3H, t, <i>J</i> = 7.1)	14.6	3.00 (3H, s)	34.4

12	8.37 (1H, s)	162.3
13	4.41 (1H, m)	68.6
14	1.25 (3H, d, $J = 7.3$)	20.7

Compound 2

Figure S15. The IR spectrum of compound 2

Figure S16. The HR-MS spectrum of compound 2

Figure S17. The ¹H spectrum of compound **2** (300 MHz in CDCl₃)

Figure S18. The ¹³C spectrum of compound **2** (75 MHz in CDCl₃)

Figure S19. The DEPT spectrum of compound **2** (in CDCl_3)

Figure S20. The ^1H - ^1H COSY spectrum of compound **2** (in CDCl_3)

Figure S21. The HMQC spectrum of compound **2** (in CDCl_3)

Figure S22. The HMBC spectrum of compound **2** (in CDCl_3)

Figure S23. The NOE spectrum of compound **2** (in CDCl_3)

Table S2. ¹H (300 MHz) and ¹³C (75 MHz) NMR data of **3-5** (δ in ppm J in Hz)

Position	3 (in CD ₃ OD)		4 (in CD ₃ OD)		5 (in CD ₃ OD)	
	δ (H)	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)
1		172.6		172.9		172.8
2						
3	3.56-3.52 (1H, m)	46.3	3.54-3.51 (2H, m)	46.6	3.59-3.55 (1H, m)	46.3
	3.49-3.46 (1H, m)				3.54-3.50 (1H, m)	
4	2.03-1.98 (1H, m)	23.4	2.06-1.99 (1H, m)	23.8	2.05-2.01 (1H, m)	23.4
	1.96-1.88 (1H, m)		1.94-1.87 (1H, m)		1.98-1.94 (1H, m)	
5	2.33-2.28 (1H, m)	29.7	2.35-2.31 (1H, m)	29.2	2.35-2.33 (1H, m)	29.7
	1.98-1.90 (1H, m)		1.96-1.87 (1H, m)		1.98-1.94 (1H, m)	
6	4.06 (1H, br. s)	61.5	4.15 (1H, br. s)	60.4	4.05 (1H, br. s)	61.7
7		167.8		169.1		167.7
8						
9	4.18 (1H, t, J = 6.9)	60.2	4.29 (1H, t, J = 7.1)	54.8	4.23 (1H, t, J = 7.1)	60.2
10	2.18-2.12 (1H, m)	25.6	1.99-1.94 (1H, m)	39.5	2.50 (1H, dq, J = 7.3)	30
			1.57-1.53 (1H, m)			
11	1.47-1.41 (1H, m)	37.2	2.06-2.01 (1H, m)	25.9	1.11 (3H, d, J = 7.3)	19
	1.35-1.29 (1H, m)					
12	0.95 (3H, t, J = 6.9)	15.7	1.01 (3H, d, J = 6.5)	23.5	0.95 (3H, d, J = 6.9)	16.8
13	1.07 (3H, d, J = 6.9)	12.6	0.97 (3H, d, J = 6.5)	22.3		

Table S3. Antifungal activities of isolated compounds **1–5** (MIC: $\mu\text{g}/\text{mL}$)

Compounds	1	2	3	4	5	Antimycin A _{2a}	Antimycin A _{7b}
MIC	>1000	>1000	>1000	>1000	>1000	31.25	31.25

Table S4. Cytotoxic activities of isolated compounds **1–5** (IC₅₀: $\mu\text{g}/\text{mL}$)

Compounds	MCF-7	SF-268	NCI-H468
1	27	>50	>50
2	>50	>50	>50
3	>50	>50	>50
4	>50	>50	>50
5	>50	>50	>50
Positive control	4	41	25.1

Figure S24. 16S rDNA of H74-21

ATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATCACTC
 TTGCAGGCATCTGTGAGGGTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTA
 TCAGCTTGTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGA
 GGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
 GTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATG
 ACGGCCCTTCGGGTTGTAAACCTCTTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCT
 GCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCA
 AGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGT
 GTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTGATACGGGCTAGCTAGAGTGTG
 GTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAAC
 ACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
 GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGAACTAGGT
 GTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGG
 GAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGGCCCCGACAAGCAGC
 GGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACA
 CCGGAAACGGCCAGAGATGGTCCGCCCTTGTGGTCGGTGTACAGGTGGTGCATGGC
 TGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTG
 TTCTGTGTTGCCAGCATGTCCTTCGGGATGATGGGGACTCACAGGAGACCGCCGGGGT
 CAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCTTATGTCTTGGGCT
 GCACACGTGCTACAATGGCCGGTACAAAGAGCAGCGATACCGTGAGGTGGAGCGAAT

CTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGG
AGTTGCTAGTAATCGCAGATC

Strain No	Names of identified species
cfcc3080	<i>Streptomyces griseoruber</i> strain cfcc3080
NBRC 12838	<i>Streptomyces antibioticus</i> strain NBRC 12838
CSSP528	<i>Streptomyces antibioticus</i> strain CSSP528
1022-257	<i>Streptomyces antibioticus</i> strain 1022-257
cfcc3075	<i>Streptomyces antibioticus</i> strain cfcc3075
cfcc3085	<i>Streptomyces antibioticus</i> strain cfcc3085
EAAG90	<i>Streptomyces antibioticus</i> strain EAAG90
NBRC 12873	<i>Streptomyces griseoruber</i> strain NBRC 12873
CSSP408	<i>Streptomyces griseoruber</i> strain CSSP408
S5	<i>Actinobacterium</i> S5
15721	<i>Streptomyces bungoensis</i> strain 15721
A316	<i>Streptomyces griseoruber</i> strain A316
HBUM174899	<i>Streptomyces longwoodensis</i> strain HBUM174899
DSM 40089	<i>Streptomyces galbus</i> strain DSM 40089
JM-R35	<i>Streptomyces caeruleatus</i> strain JM-R35
JCM 3373	<i>Streptomyces lasaliensis</i> strain JCM 3373
NBRC 12849	<i>Streptomyces cellostaticus</i> strain NBRC 12849
BCCO 10_1548	<i>Streptomyces curacoi</i> strain BCCO 10_1548
1043	<i>Streptomyces panayensis</i> strain 1043
NBRC 15711	<i>Streptomyces bungoensis</i> strain NBRC 15711