SUPPLEMENTARY MATERIAL

Callyptide A, a New Cytotoxic Peptide from the Red Sea Marine Sponge *Callyspongia* species

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In the course of our continuing efforts to allocate bioactive secondary metabolites from Red Sea marine Invertebrates, we have investigated the sponge *Callyspongia* species. The cytotoxic dichloromethane fraction of the methanolic extract of the sponge afforded a new cytotoxic peptide named callyptide A (**1**). Its structure was determined by extensive 1D and 2D NMR (COSY, HSQC and HMBC) studies and high-resolution mass spectral determination. The configuration of the amino acids was determined by Marfey's analysis. Callyptide A was found to exhibit growth inhibitory activity when tested against different cancer cell lines.

Keywords: Red Sea sponge; *Callyspongia* species; Callyptide A; Cancer cell lines; Cytotoxic activity.

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Figure S1. ^1H NMR spectrum of compound **1** (500 MHz, $\text{DMSO-}d_6$).

Figure S2. ^{13}C NMR spectrum of compound **1** (125 MHz, $\text{DMSO-}d_6$).

Figure S3. ^1H - ^1H COSY spectrum of compound **1**.

Figure S4. HSQC spectrum of compound **1**.

Figure S5. HMBC spectrum of compound **1**.

Figure S6. Substructures A-D, ^1H - ^1H COSY, and selected HMBC correlations of **1**.

Table S1. NMR spectroscopic data of compound 1 (DMSO-*d*₆)

Amino acid	C#	δ_{H} m (<i>J</i> in Hz)	δ_{C}	HMBC
Thr¹	1	-	174.2 C	-
	2	4.32 dd (10.1, 6.9)	50.7 CH	1, 4, 5
	3	3.71 dd (10.1, 6.8)	71.8 CH	1, 2
	4	0.88 d (6.8)	21.4 CH ₃	2, 3
	NH	8.33 d (6.9)	-	2, 3, 5, 6
Ala²	5	-	172.5 C	-
	6	4.29 dq (7.9, 7.1)	47.8 CH	5, 8
	7	1.16 d (7.1)	18.0 CH ₃	5, 6, 8
	NH	7.23 d (7.9)	-	5, 8
Leu³	8	-	171.1 C	-
	9	3.85 dd (7.9, 6.9)	53.1 CH	8, 11, 14
	10	1.51 m	42.7 CH ₂	9, 12, 13
	11	1.47 m	27.2 CH	9, 12, 13
	12	0.87 d (6.7)	22.6 CH ₃	10, 11
	13	0.87 d (6.7)	22.6 CH ₃	10, 11
	NH	7.38 d (7.9)	-	8, 9, 14, 15
Tyr⁴	14	-	170.4 C	-
	15	4.58 dt (8.6, 6.5)	55.2 CH	14, 17, 23
	16	2.86 dd (13.5, 6.5) 2.81 m	36.3 CH ₂	14, 18, 22
	17	-	128.3 C	-
	18, 22	7.07 d (7.9)	130.1 CH	17, 19, 20, 21
	19, 21	6.61 d (7.9)	114.8 CH	17, 18, 20, 22
	20	-	157.4 C	-
	NH	7.33 d (8.6)	-	16, 23, 24
Phe⁵	23	-	166.4 C	-
	24	4.52 dd (7.6, 6.8)	55.5 CH	23, 25, 26, 32
	25	2.94 dd (13.7, 6.8) 2.92 m	36.5 CH ₂	23, 26, 27, 31
	26	-	135.4 C	-
	27, 31	7.19 m	129.1 CH	25, 26, 28, 29, 30
	28, 30	7.10 m	128.9 CH	26, 27, 29, 31
	29	7.14 m	127.9 CH	27, 28, 30, 31
	NH	8.43 d (7.6)	-	25, 32, 33
Ile⁶	32	-	171.6 C	-
	33	4.32 dd (7.1, 6.9)	50.7 CH	34, 37, 38
	34	1.83 m	39.3 CH	32, 36, 37
	35	1.65 m 1.35 m	24.1 CH ₂	36, 37
	36	0.79 t (7.1)	9.2 CH ₃	34, 37
	37	0.85 d (6.9)	21.4 CH ₃	33, 36
	NH	8.35 d (6.9)	-	32, 38, 39
3-OHLeu⁷	38	-	176.3 C	-
	39	4.50 dd (10.4, 7.8)	55.6 CH	38, 40, 41, 44
	40	4.75 dd (10.4, 7.1)	74.5 CH	38, 41, 42, 43
	41	1.72 m	28.8 CH	39, 42, 43
	42	0.84 d (6.9)	23.2 CH ₃	40, 41
	43	0.84 d (6.9)	23.2 CH ₃	40, 41
	NH	8.41 d (7.8)	-	38, 44, 45
Tyr⁹	44	-	170.7 C	-

45	4.58 dt (8.6, 6.5)	55.2 CH	1, 47
46	2.86 dd (13.5, 6.5) 2.81 m	36.3 CH ₂	47, 48, 52
47	-	128.0 C	-
48, 52	7.04 d (7.9)	130.1 CH	49, 50, 51
49, 51	6.60 d (7.9)	114.8 CH	47, 48, 50, 51
50	-	157.6 C	-
NH	7.33 d (8.6)	-	1, 2, 44, 46