SUPPLEMENTARY MATERIAL

Monanchoxymycalin C with Anticancer Properties, New Analogue of Crambescidin 800 from the Marine Sponge Monanchora pulchra

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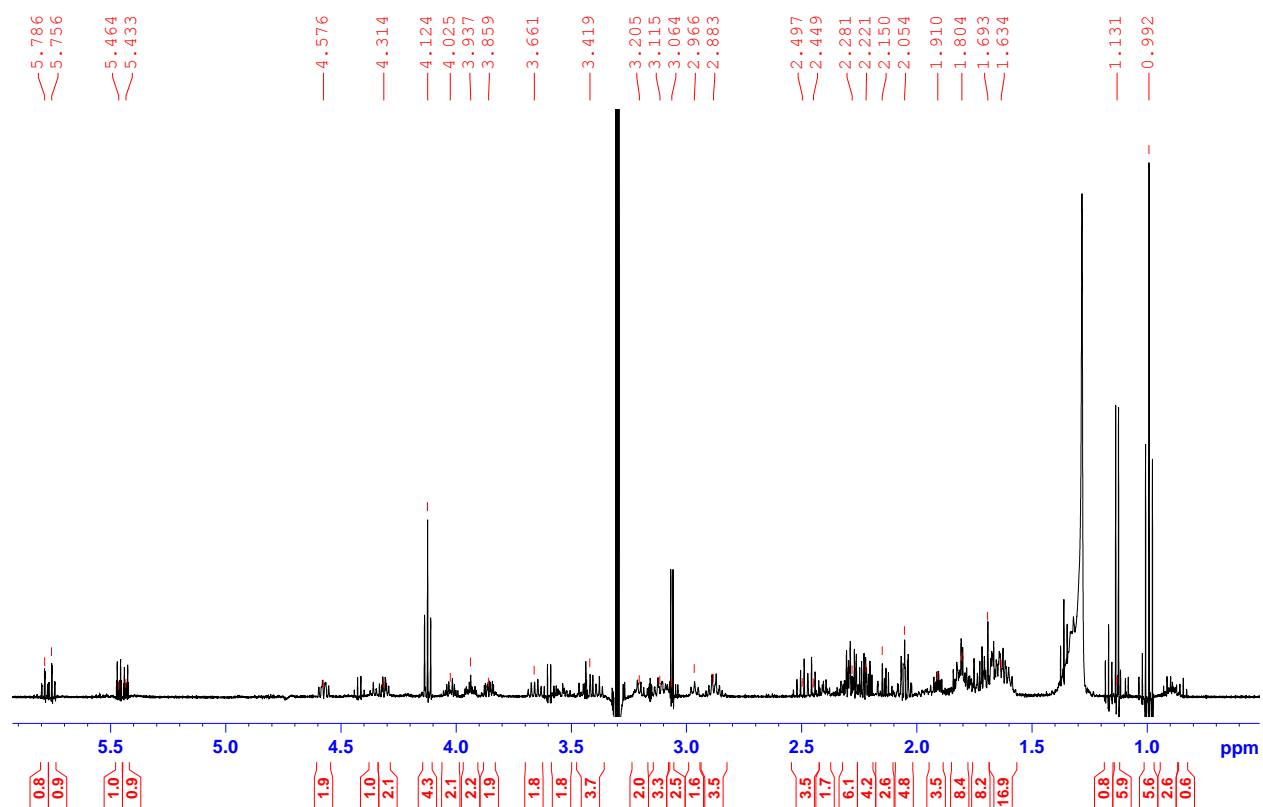* To whom correspondence should be addressed: Email: **makarieva@piboc.dvo.ru**

Abstract: A new pentacyclic guanidine alkaloid, monanchoxymycalin C (**1**) was isolated from marine sponge *Monanchora pulchra* along with the known monanchoxymycalin A (**2**). The structure of **1** was elucidated on the basis of spectroscopic data. Monanchoxymycalin C exhibits cytotoxic activity against human cancer HeLa cells at low micromolar concentrations, induces apoptosis-related death of malignant cells and inhibits cancer cell colony formation. In addition, synergistic and additive effects have been observed in combination with cisplatine.

Keywords: pentacyclic guanidine alkaloids; marine sponge; cytotoxic activity; apoptosis
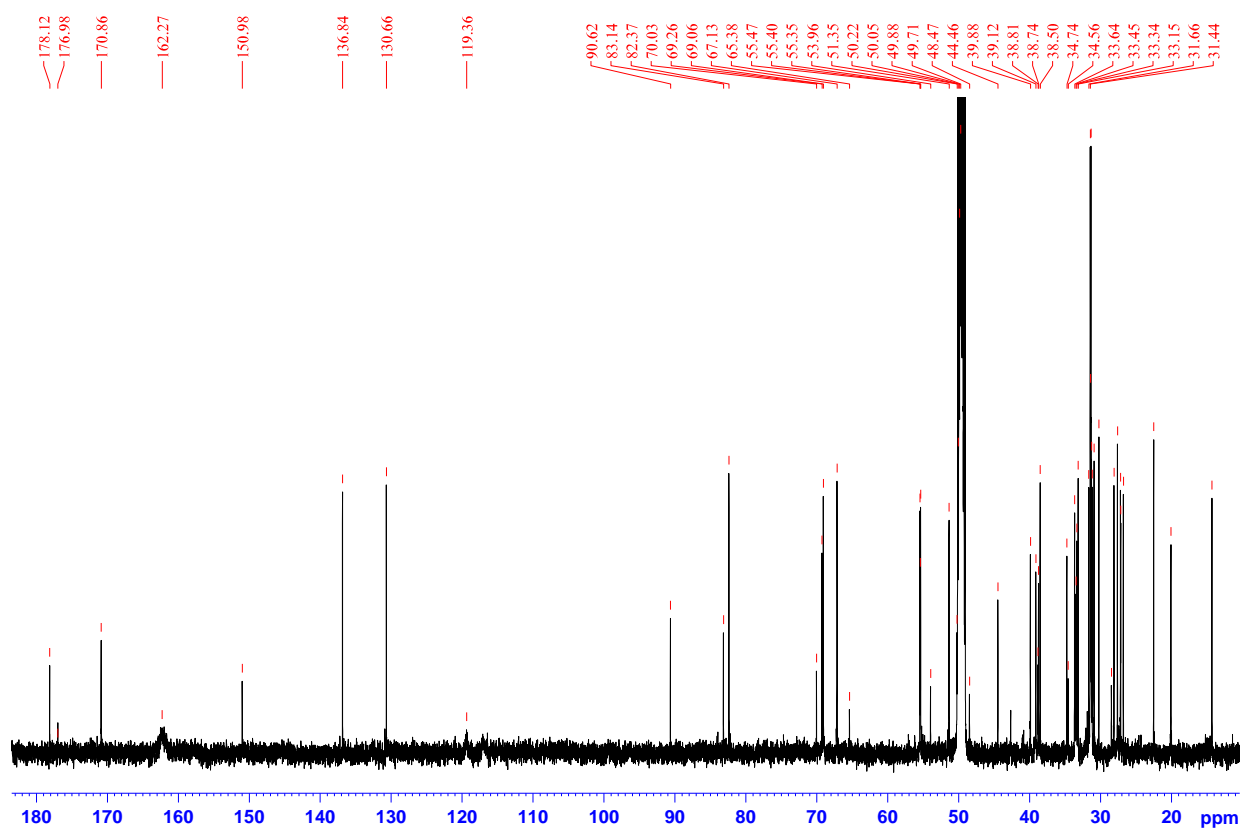
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Figure S4. HSQC spectrum of compound 1 in CD$_3$OD (500/125 MHz)

Figure S5. HMBC spectrum of compound 1 in CD₃OD (500/125 MHz)

Figure S6. HRESIMS spectrum of compound 1

Figure S7. Effect of **1** on induction of apoptosis and cell cycle progression. HeLa cells were treated with compounds for 48 h. **a**, FACS analysis of DNA fragmentation. The amount of apoptotic cells (sub-G1 population) was quantified using the Cell Quest Pro software. Cisplatin (Cis) was used as a positive control. **b**, Western blotting analysis of protein extracts of HeLa cells treated with **1**. Cells treated with 12.5 μM of cisplatin (Cis) were used as positive control. **c**, FACS analysis of cell cycle progression. HeLa cells were treated with 0.625 μM of **1** for 48 h. Cell cycle phase distribution was analyzed and quantified using the Cell Quest Pro software.

Figure S8. Effect of **1** in combination with cisplatin on viability of HeLa cells and the effect of **1** on cancer cell colony formation. **a**, Cell viability was examined by the MTS assay. Cells were co-treated with different concentrations of the single substances or their combination for 48 h at non-constant molar ratio. The combinational index (CI) values were calculated with CompuSyn software. The ratio of the substances were C(**1**) : C(Cis) = 1 : 5 or 1 : 2.5. The concentrations of the individual compounds used in the drug combination as well as CI values are presented in Table S2. **b**, Colony formation assay. HeLa cells were treated with the drugs for 48 h, then 100 alive cells were plated in 6-well plates and incubated for 10 days. Cell colonies were fixed, stained, and counted. Significant difference from the control is shown as follows: *** $p < 0.001$ (Student's t-test)

Table S1: ^1H and ^{13}C NMR spectroscopic data for compounds **1** and **2** in CD_3OD^a

Carbon	Monanchoxymycalin C (**1**)				Monanchoxymycalin A (**2**)	
n	$\delta_C{}^b$, mult	δ_H, mult (*J* in Hz)	H–H COSY	HMBC	$\delta_C{}^b$, mult	δ_H, mult (*J* in Hz)
1	14.3, CH_3	1.00 t (7.5)	2	2, 3	14.3, CH_3	0.99 t (7.5)
2a	26.8, CH_2	2.06 m	1, 3	1, 3, 4	26.8, CH_2	2.05 m
2b						
3	136.8, CH	5.78 dt (6.3, 15.3)	2, 4	1, 2, 4, 5	136.8, CH	5.77 dt (6.3, 15.3)
4	130.7, CH	5.45 ddt (1.5, 7.4, 15.3)	3, 5	2, 5	130.7, CH	5.45 ddt (1.5, 7.4, 15.3)
5	82.3, CH	4.58 brq (7.2)	4, 6	3	82.4, CH	4.57 brq (7.2)
6a	33.1, CH_2	1.81 m	5, 7	4, 7, 8	33.2, CH_2	1.81 m
6b		2.23 m				2.23 m
7a	38.4, CH_2	2.15 m	6	5, 6, 8	38.5, CH_2	2.15 m
7b		2.20 m				2.22 m
8	90.6, C				90.6, C	
9a	39.9, CH_2	1.69 m	10	8, 10, 11	39.9, CH_2	1.71 m
9b		2.27 m				2.31 m
10	55.4, CH	4.03 m	9, 11	8, 11, 21	55.4, CH	4.02 m
11a	31.7, CH_2	1.64 m	10, 12	12, 13	31.7, CH_2	1.67 m
11b		2.29 m				2.29 m
12a	28.1, CH_2	1.76 m	11, 13	14	28.1, CH_2	1.76 m
12b		2.29 m				2.28 m
13	55.5, CH	4.32 m	12, 14	12, 14	55.6, CH	4.30 m
14	51.4, CH	3.06 d (5.0)	13	13, 15, 22	51.4, CH	3.08 d (5.0)
15	83.1, C				83.1, C	
16	33.3, CH_2	1.69 m			33.4, CH_2	1.67 m
17	20.0, CH_2	1.81 m	18		20.1, CH_2	1.81 m
18a	33.6, CH_2	1.28 m	17, 19		31.7, CH_2	1.26 m
18b		1.69 m				1.66 m
19	69.0, CH	3.86 m	18, 20	15, 17, 20	72.3, CH	3.72 m
20	22.5, CH_3	1.14 d (6.1)	19	15, 18, 19	39.5, CH_2	1.39 m 1.46 m
21	151.0, C				151.0, C	
22	170.8, C				170.9, C	
23	67.1, CH_2	4.13 t (6.6)	24	22, 24	67.1, CH_2	4.12 t (6.6)
24	30.2, CH_2	1.64 m	23	23	30.2, CH_2	1.64 m
25-35	31.2-31.5	1.21-1.32 brs			31.2-31.5	1.21-1.32 brs
36	27.2, CH_2	1.61 m	37	37, 38	27.3, CH_2	1.61 m
37a	34.7, CH_2	2.44 m	36	38	34.7, CH_2	2.47 m
37b		2.48 m				
38	178.1, C				178.1, CH_2	
39a	44.5, CH_2	3.40 m	40	38, 42	44.4, CH_2	3.40 m
39b		3.66 m				3.66 m
40	27.1, CH_2	1.91 m	39, 41		27.1, CH_2	1.90 m
41	38.7, CH_2	2.89 m	40	39	38.5, CH_2	2.88 m
42a	55.3, CH_2	3.27 m	43		55.4, CH_2	3.28 m

42b		3.43 m			3.44 m
43	69.3, CH	3.94 m	42, 44	69.3, CH	3.93 m
44a	33.4, CH$_2$	1.71 m	43, 45	33.4, CH$_2$	1.69 m
44b		1.83 m			1.82 m
45	39.1, CH$_2$	3.12 m	44	39.1, CH$_2$	3.12 m
46a				20.1	1.32 m
46b					1.39 m
47				14.8	0.87 t (7.3)

[a] Spectra were recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR.
[b] ^{13}C NMR assignments supported by HSQC and HMBC data.

Table S2. The concentrations of the individual compounds used in the drug combination as well its effect (Fa) and combinational index (CI) values. Combinational index were calculated with CompuSyn v.1.0. Software (ComboSyn, Inc., Paramus, NJ, USA).

Dose compound 1 (µM)	Effect (Fa)
0.625	0.404
1.25	0.64
2.5	0.695
5.0	0.749
0.625	0.387
1.25	0.62
2.5	0.728
5.0	0.749

Dose of Cis (µM)	Effect (Fa)
3.125	0.546
6.25	0.764
12.5	0.952
25.0	0.977
1.5625	0.285
3.125	0.478
6.25	0.767
12.5	0.957
3.125	0.498
6.25	0.723
12.5	0.928
25.0	0.976
1.5625	0.241
3.125	0.437
6.25	0.741
12.5	0.946

Combination of compound 1 with cisplatin			
Dose of compound 1 (µM)	Dose of Cis (µM)	Effect (Fa)	CI
0.625	3.125	0.702	0.86435
0.625	1.5625	0.549	1.02154
1.25	6.25	0.902	0.67932
1.25	3.125	0.781	0.76085
2.5	12.5	0.959	0.77625
2.5	6.25	0.913	0.68044
0.625	3.125	0.6	1.2393
0.625	1.5625	0.489	1.31216
1.25	6.25	0.89	0.7367
1.25	3.125	0.731	0.95768
2.5	12.5	0.952	0.85468
2.5	6.25	0.888	0.8267
5.0	25.0	0.968	1.33845
5.0	12.5	0.942	1.02032

| 5.0 | 25.0 | 0.978 | 1.07532 |
| 5.0 | 12.5 | 0.95 | 0.92353 |

Figure S9. Foto of the sponge *Monanchora pulchra*