Chemical constituents screening of the marine sponge *Neopetrosia proxima* and the octocoral *Briareum stechei*

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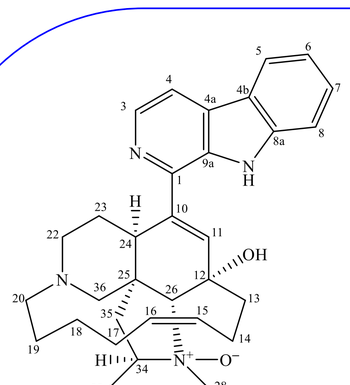
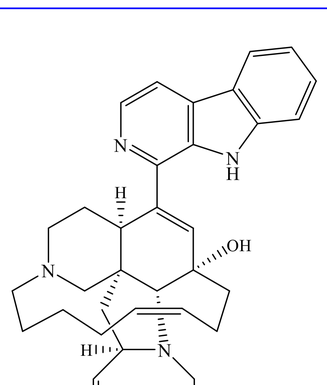
Chemical constituents screening of two marine invertebrates led to the isolation of six macrocyclic alkaloids, including a novel manzamine, manzamine A 27-*N*-oxide (**1**), as well as six known analogues, manzamines A, E, and F (**2**–**4**), 8-hydroxymanzamine A (**5**), and 8-hydroxymanzamine A hydrochloride (**6**) from the Formosan sponge *Neopetrosia proxima*. Furthermore, 12 briarane-type diterpenoids were isolated from the Okinawan coral *Briareum stechei*, including five new briaranes, briarenols U (**7**) and V (**8**), briastecholides A–C (**9**–**11**), together with seven known metabolites, briaexcavatolide E (**12**), brianolide (**13**), briarenol R (**14**), briarenolide S (**15**), solenolides B, C, and E (**16**–**18**). The structures of **1**–**18** were determined based on spectroscopic data, **1** was proved to be the first manzamine alkaloid possessing the 27-*N*-oxide moiety, stereochemistry of solenolide B (**16**) was revised, and the absolute configurations of **1**, **6**, **13** and **17** were established using single X-ray diffraction analysis. Manzamines **1**–**3** showed cytotoxicity towards a panel of tumor cells and briaranes **7**–**18** were evaluated for their *in vitro* inflammatory activity in LPS-induced RAW 264.7 macrophage cells by suppressing the expression of iNOS and COX-2 proteins.



1*



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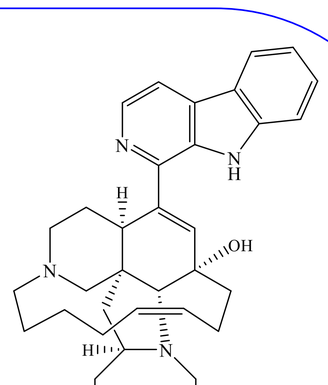

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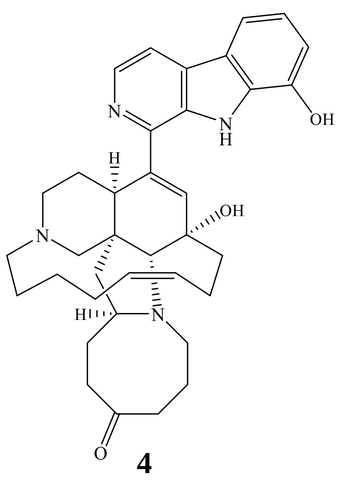

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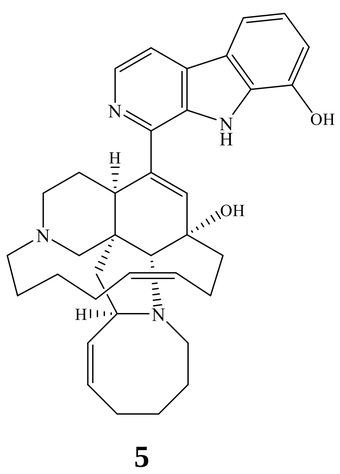

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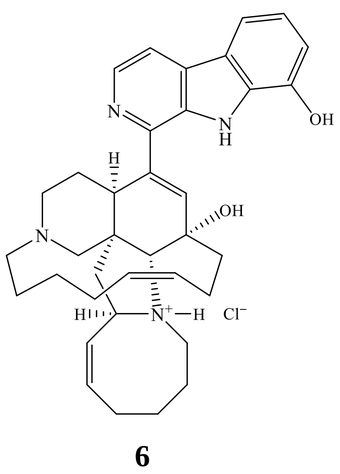

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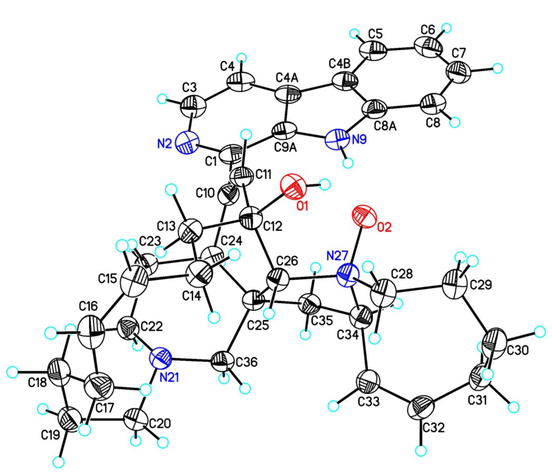
Figure 1. Structures of manzamine A 27-*N*-oxide (**1**), manzamines A (**2**), E (**3**), and F (**4**), 8-hydroxymanzamine A (**5**), and 8-hydroxymanzamine A hydrochloride (**6**). * New.



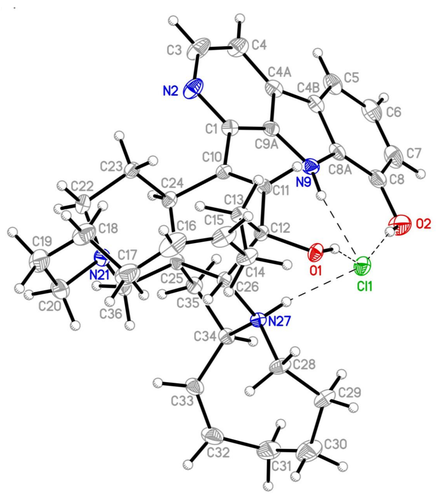


Figure 2. Computer-generated ORTEP diagram of **1** (left) and **6** (right).

Table 1. Effects of briaranes **7**–**12** and **14**–**18** on LPS-induced pro-inflammatory iNOS and COX-2 protein expressions in macrophages.

Compound/ Treatment (10 μM)	iNOS	COX-2	*β*-Actin	*n*
	Expression (% of LPS)			
Control	2.28 ± 0.04	0.76 ± 0.05	99.12 ± 3.19	4
Vehicle	100.00 ± 1.75	100.00 ± 1.86	100.00 ± 4.84	4
7	111.92 ± 12.23	149.29 ± 4.27	93.32 ± 6.80	3
8	80.20 ± 8.09	100.89 ± 2.58	94.59 ± 5.49	3
9	88.27 ± 0.25	101.84 ± 3.07	100.43 ± 5.32	3
10	86.46 ± 3.85	100.45 ± 2.59	103.13 ± 2.46	3
11	79.30 ± 3.13	100.39 ± 3.30	104.24 ± 5.58	3
12	88.55 ± 4.48	103.09 ± 3.83	106.11 ± 5.08	4
14	87.52 ± 2.84	105.33 ± 6.10	98.29 ± 3.35	4
15	66.49 ± 3.41	112.31 ± 5.76	99.33 ± 4.40	4
16	90.81 ± 8.40	99.16 ± 2.83	101.48 ± 4.15	4
17	88.87 ± 2.87	94.69 ± 3.24	101.12 ± 4.37	4
18	94.97 ± 5.24	99.52 ± 2.02	104.45 ± 4.43	4
Dexamethasone	53.21 ± 0.78	12.72 ± 0.35	107.55 ± 0.37	4

Data were normalized to those of cells treated with LPS only and expresses as the mean ± SEM (n = 4~5). Dexamethasone was used as positive control.

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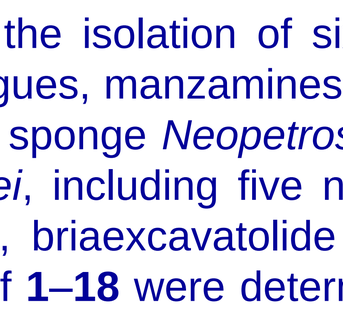
8*

9*



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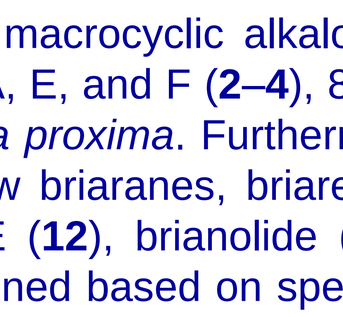

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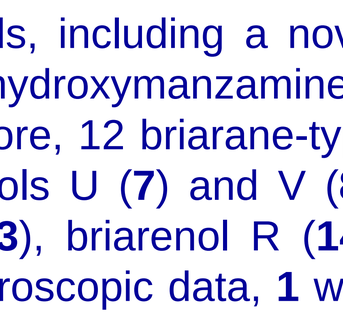

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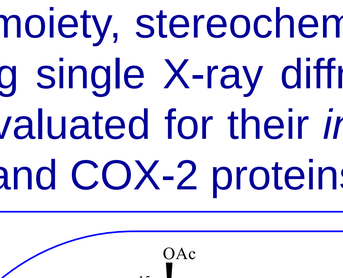

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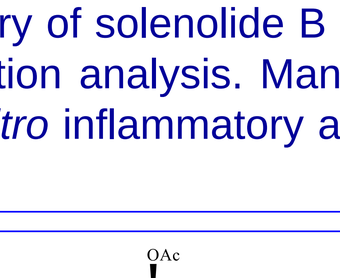

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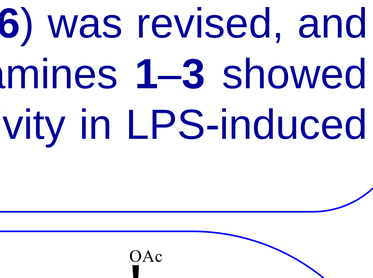

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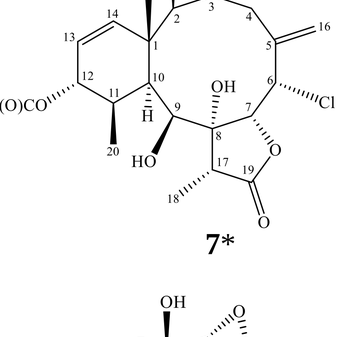

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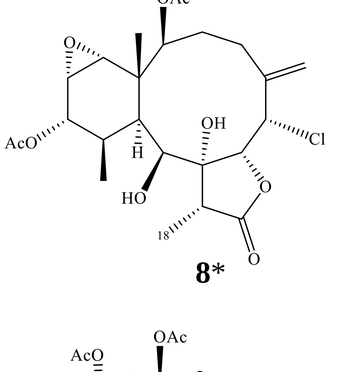

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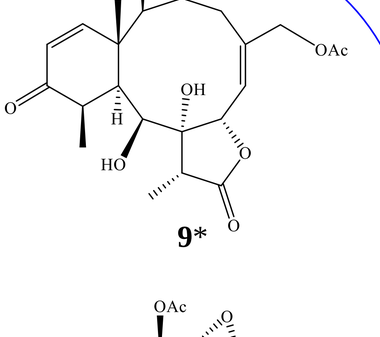

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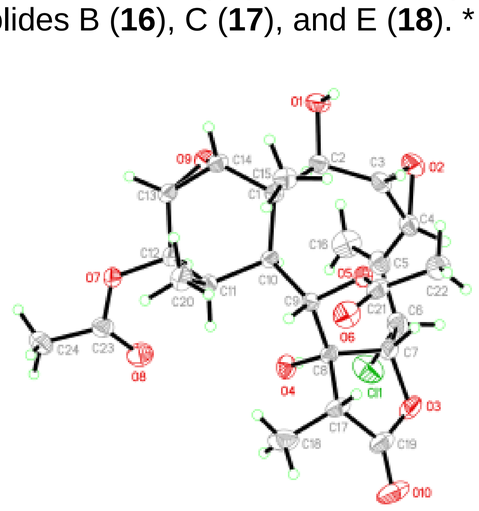
Figure 3. Structures of briarenols U (**7**) and V (**8**), briastecholides A–C (**9**–**11**), briaexcavatolide E (**12**), brianolide (**13**), briarenol R (**14**), briarenolide S (**15**), solenolides B (**16**), C (**17**), and E (**18**). * New compound.



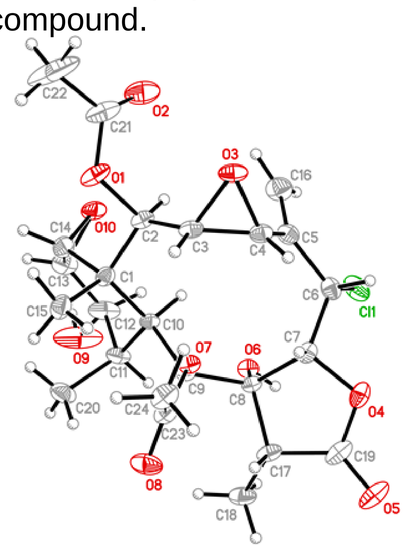


Figure 4. Computer-generated ORTEP diagram of **13** (left) and **17** (right).

Table 2. Cytotoxic effects of **1**–**3** on tumor cells.

Compound/ Treatment	Hep3B	SC-M1	MCF-7
	IC_{50} (μM)		
1	10.1 ± 3.1	11.7 ± 4.5	10.6 ± 2.7
2	3.8 ± 1.3	3.7 ± 1.2	4.1 ± 1.7
3	5.8 ± 0.7	5.6 ± 0.4	4.2 ± 0.9
Doxorubicin*	0.09 ± 0.02		
Taxol*		0.06 ± 0.1	
Tamoxifen*			10.7 ± 4.3

*Positive control. Hep3B: human hepatocellular carcinoma cells. SC-M1: human stomach tumor cells. MCF-7: human breast cancer cells.