Dysynosin A: A Novel inhibitor of Factor VIIa and Thrombin from a new genus and species of the Australian sponge of the family Dysideidae

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Supporting Information

The methanol extract from a new genus and species of sponge¹ of the family Dysideidae inhibited Factor VIIa, (IC₅₀ 5µg/mL). Solvent partition between DCM and water resulted in concentration of activity in the water fraction. Bioassay guided fractionation of the water fraction by C18 MPLC followed by successive RP HPLC on C18, CN and phenyl bonded silica afforded 95 mg (0.014%) of dysynosin A **1** as the bioactive component.

Dysynosin A (**1**): [α]_D +30.7 (c 0.1 MeOH); UV (MeOH) $\lambda_{\max}$ 210 nm (ϵ) 114,815; was assigned a molecular formula C₂₆H₄₄N₆O₁₀S by ESIMS mass measurement of the MNa⁺ ion (655.2707: requires 655.2732).

The crystal belongs to monoclinic space group C2 with cell constants of a = 69.89 Å, b = 72.31 Å, c = 69.31 Å, $\alpha = \gamma = 90^\circ$, $\beta = 99.57^\circ$ with one protein molecule per asymmetric unit.

The X-ray data to 1.80 Å resolution was collected on the high brilliance beamline ID2 at ESRF Grenoble, France using MAR-II imaging plate system, MAR Research, Hamburg, Germany. The data was reduced and scaled using DENZO (2) and SCALA from the CCP4 (3) package.

Statistics for X-ray data collection and refinement.

X-ray wavelength (Å)	0.97
No of measurements	60511
No of unique reflections	27588
Data completeness	90.6 (68.9)*
R _{merge} ^a	0.062 (0.184)*
No of solvent mol.	170
Resolution range (Å)	19.2-1.80
R _{cryst} ^b / R _{free}	0.199 / 0.246

^aR_{merge} = $\sum_i \sum_j (I(h,i) - \langle I(h) \rangle) / \sum_i \sum_j I(h,i)$ where $I(h,i)$ is the intensity value of the i^{th} measurement of h and $\langle I(h) \rangle$ the corresponding mean value of h for all i measurements of h .

^bR_{cryst} = $\sum_{hkl} (|F_o| - |F_c|) / \sum_{hkl} |F_o|$, $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes, respectively. the numbers in brackets are related to the data in the highest resolution shell, i.e. from 1.90 to 1.80 Å

¹ This new genus and species are currently in press and in preparation, respectively, with sources: [genus name] Cook, S.de C., Bergquist, P.R. (2002) Family Dysideidae. In: Hooper, J.N.A., Van Soest, R.W.M. (eds) "Systema Porifera. A Guide to the Classification of Sponges" (Kluwer Academic/Plenum Publishers, New York). [species name] Hooper, J.N.A., Schlacher, M., Carroll A.R. (in prep.) Cryptic Dysideidae (Porifera, Demospongiae) from the Great Barrier Reef, Australia.

¹H (600 MHz) and ¹³C (150 MHz) NMR data for the major conformer of dysinosin A (1) in d₆-DMSO

Atom	¹³ C ppm	¹ H ppm (mult <i>J</i> (Hz))
1a	66.2 (2)	3.75 (dd, 7.8, 10.8)
1b		3.95 (dd, 3.0, 10.8)
2	80.3 (1)	3.89 (dd, 3.0, 7.8)
2-OCH ₃	57.2 (3)	3.30 (s)
3	169.7 (0)	-
4	-	7.96 (d, 7.2)
5	49.3 (1)	4.45 (ddd, 3.4, 7.2, 10.2)
6a	40.0 (2)	1.27 (ddd, 3.4, 9.6, 13.2)
6b		1.55 (m)
7	24.2 (1)	1.50 (m)
8	21.3 (3)	0.90 (d, 6.6)
9	23.2 (3)	0.89 (d, 6.6)
10	170.2 (0)	-
11	-	-
12	54.0 (1)	4.07 (td, 6.6, 11.4)
13a	27.8 (2)	1.95 (m)
13b		
14	68.5 (1)	3.69 (dddd, 3.4, 3.4, 3.4, 3.4)
14-OH	-	4.76 (d, 3.4)
15	67.0 (1)	3.56 (dddd, 2.9, 3.4, 3.4, 3.4)
15-OH		4.71 (d, 2.9)
16a	26.7 (2)	1.50 (m)
16b		1.90 (m)
17	35.2 (1)	2.15 (m)
18a	33.3 (2)	2.08 (ddd, 7.2, 8.4, 12.0)
18b		2.30 (ddd, 10.2, 12.0, 12.0)
19	59.8 (1)	4.04 (dd, 8.4, 12.0)
20	171.2 (0)	-
21	-	7.36 (t, 6.6)
22a	36.4 (2)	3.08 (qd, 6.6, 12)
22b		3.26 (qd, 6.6, 12)
23a	28.2 (2)	2.25 (m)
23b		2.27 (m)
24	136.0 (0)	-
25	119.1 (1)	5.60 (bs)
26	54.8 (2)	4.15 (m)
27	-	-
28	154.1 (0)	-
29	-	7.20 (bs)
30	-	7.20 (bs)
31	55.2 (2)	4.12 (m)

no. of attached protons determined from ¹³C and DEPT experiments