

Supplementary Information

Bioprospecting deep-sea Actinobacteria for novel anti-infective natural products

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Methods

The *Clostridium difficile* cell lysis assay was performed as described previously (Ericsson et al., 1960). A single colony UK6 was inoculated into BHIS and cultured at 37°C for 24 h; afterwards, 200 µL of UK6 were mixed with 20 mL of BHIS to make UK6-seeded plates. After solidification, 1.0 µL aliquots of vancomycin or M864 extracts WG1-60-60 and WG1-60-61 with different concentrations, 1 or 2 mg/mL, respectively, were dropped onto the plate. Plates were kept at 37°C in an anaerobic chamber for 24-48 h until the inhibition zone is clearly seen. Methanol is included as a negative control.

References

- Ericsson, B.H., Tunevall, G., and Wickman, K. (1960). The paper disc method for determination of bacterial sensitivity to antibiotics: relationship between the diameter of the zone of inhibition and the minimum inhibitory concentration. *Scand. J. Clin. Lab. Invest.* 12, 414-43 422.
- Sfanos, K., Harmody, D., Dang, P., Ledger, A., Pomponi, S., McCarthy, P., and Lopez, J. (2005). A molecular systematic survey of cultured microbial associates of deep-water marine 46 invertebrates. *Syst. Appl. Microbiol.* 28, 242-264. doi: 10.1016/j.syapm.2004.12.002

Results

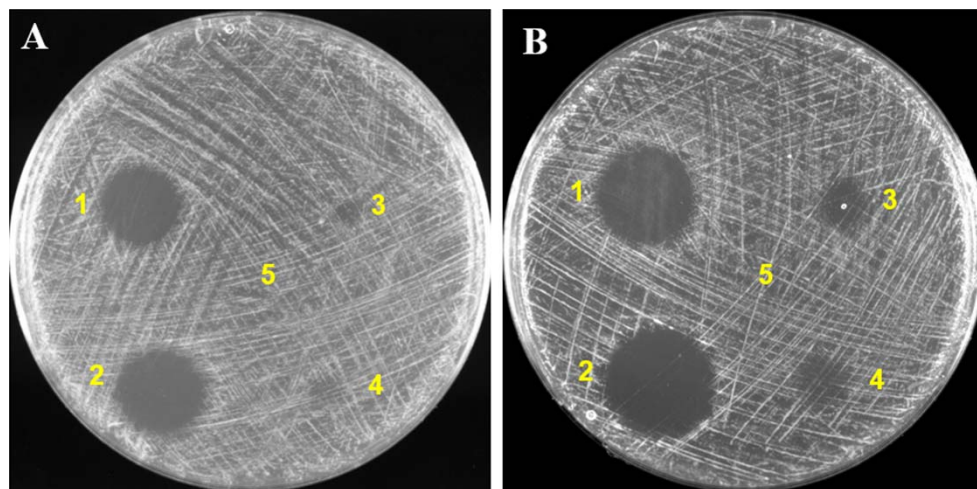


Figure S1. Antibacterial activity of the compound **1** using cell lysis assay. Moderate inhibition of *C. difficile* growth was observed. An aliquot of 2 μ L sample solution at either 3.2 mg/mL (**A**) or 6.4 mg/mL (**B**) was dropped onto the UK6-seeded plate. 1, 2: vancomycin; 3, 4: compound **1**; 5: methanol. No inhibition of other bacterial pathogens, *S. aureus*, MRSA and *P. aeruginosa*, are observed.

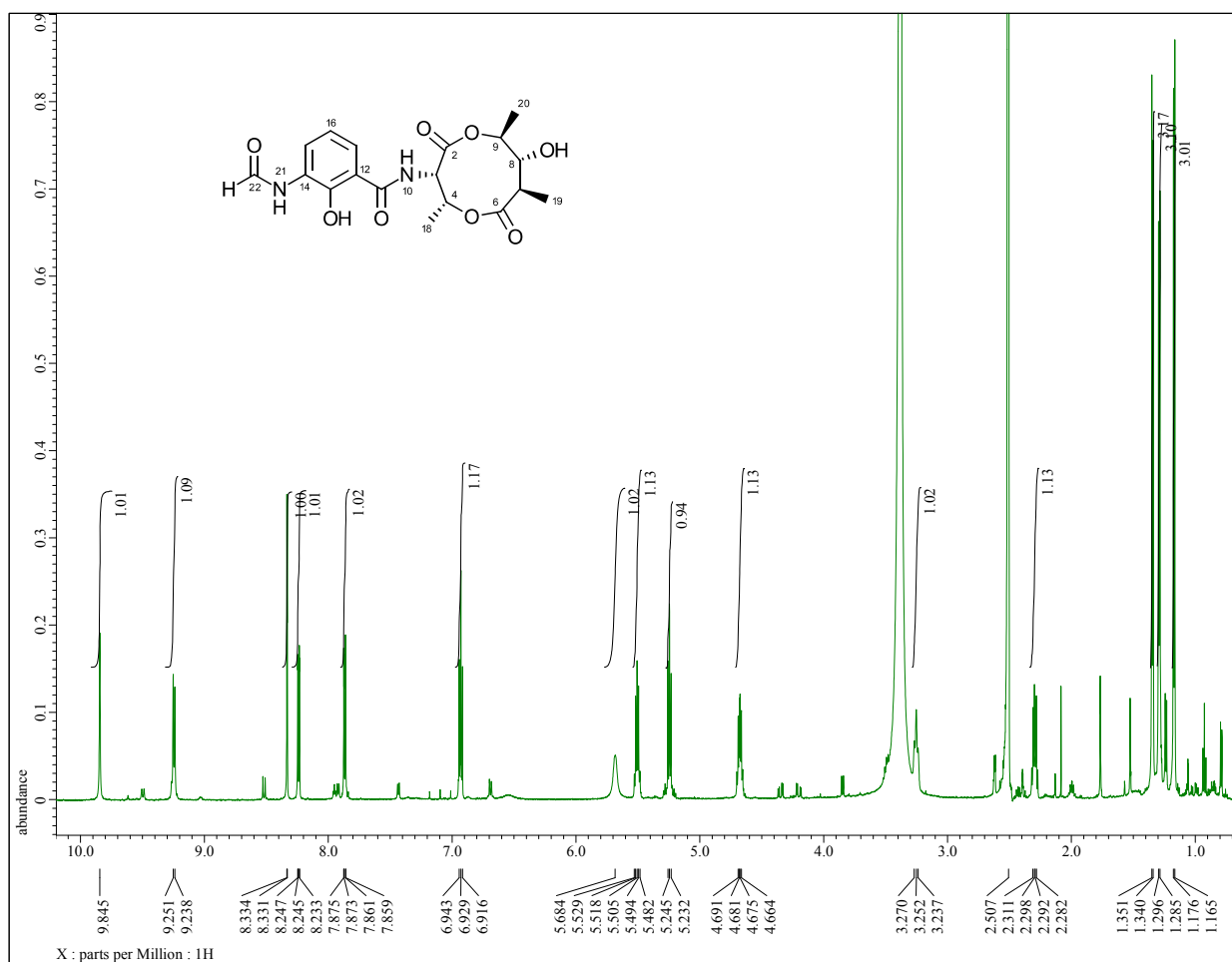


Figure S2. ^1H NMR spectrum of compound **1** in $\text{DMSO-}d_6$.

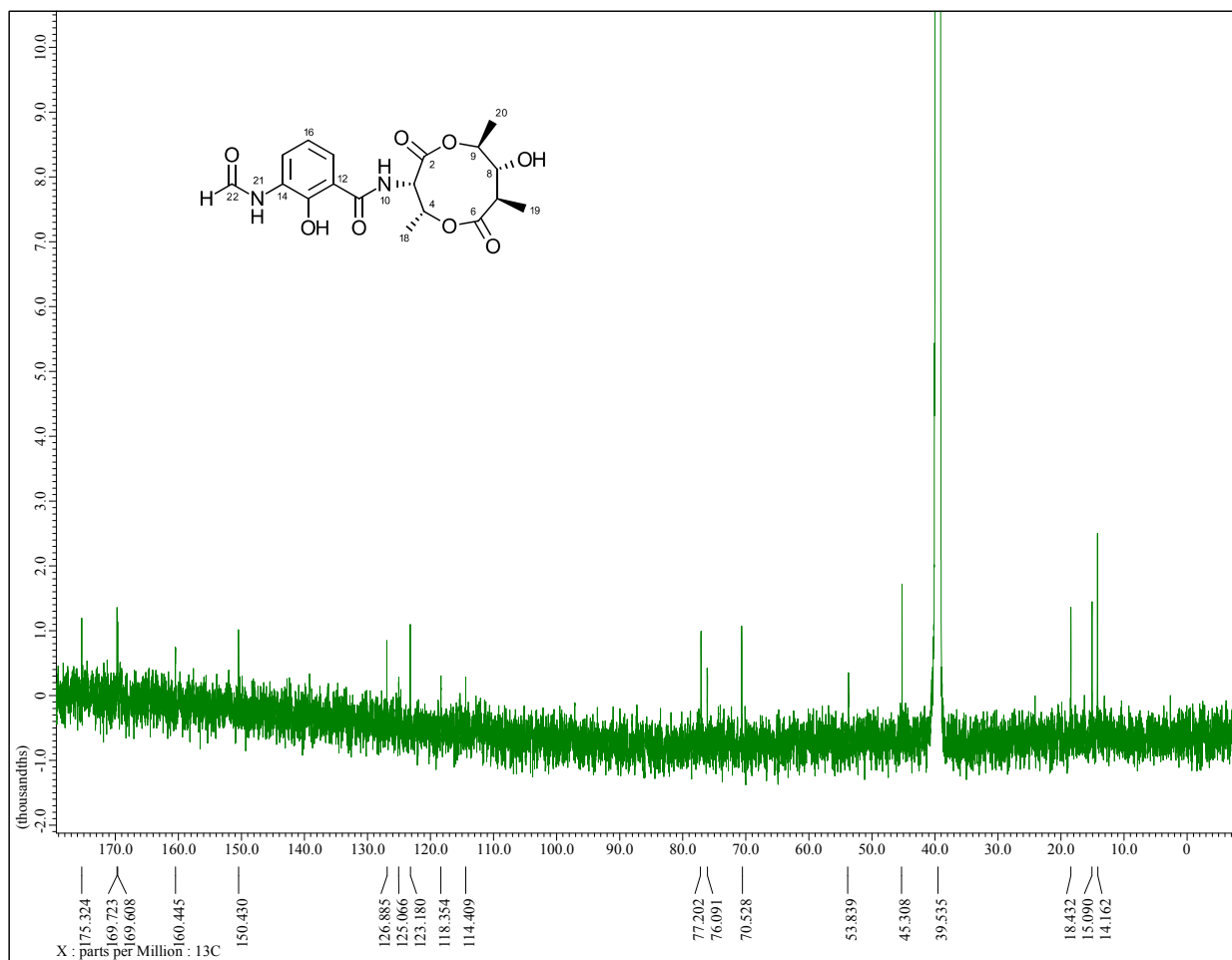


Figure S3. ^{13}C NMR spectrum of compound **1** in $\text{DMSO-}d_6$.

Table S1. Strains (50) used in this study, taxonomy and their sponge origin.

No.	Strain	Taxonomy *	Accession No.	Sponge origin (Year**)
1	E432	<i>Pseudonocardia</i> sp.		<i>Sarcotagus</i> sp. or <i>Smenospongia</i> sp. (1992)
2	E677	<i>Streptomyces</i> sp.		<i>Ircinia felix</i> (1992)
3	H882	<i>Pseudonocardia</i> sp. SH2		<i>Thrinacophora funiformis</i> (1995)
4	J378	<i>Nocardiopsis</i> sp.		<i>Theonella</i> sp. (1996)
5	J379	<i>Streptomyces</i> sp.	AY371434	<i>Theonella</i> sp. (1996)
6	J380	<i>Nocardiopsis alborubida</i>	AY368532	<i>Theonella</i> sp. (1996)
7	J653	<i>Rhodococcus opacus</i>		<i>Spongosorites</i> sp. (1996)
8	J855	<i>Gordonia-like</i> sp.	AY368507	<i>Spongosorites</i> sp. (1996)
9	K105	<i>Pseudonocardia</i> sp. HUST013		<i>Discodermia</i> sp. (1996)
10	K137	<i>Pseudonocardia</i> sp. AL041005-10		<i>Discodermia</i> sp. (1996)
11	K164	<i>Pseudonocardia</i> sp. HUST013		<i>Discodermia</i> sp. (1996)
12	K375	<i>Tsukamurella</i> sp. FXJ1.531		<i>Hexactinellida</i> + <i>Zoanthidea</i> (2000)
13	K688	<i>Rhodococcus ruber</i> Er I B2.3		<i>Discodermia</i> sp. (1997)
14	L128	<i>Streptomyces</i> sp. M7		<i>Discodermia</i> sp. (1998)
15	L271	<i>Rhodococcus</i> sp. CNY-462		<i>Forcepia</i> sp. (1999)
16	L733	<i>Streptomyces</i> sp.2		<i>Axinellida</i> (1999)
17	M864	<i>Salinispora</i> sp.		<i>Oceanapiidae</i> (1993)
18	N197	<i>Streptomyces</i> sp. JVCH105X		<i>Forcepia</i> sp. (1999)
19	N201	<i>Streptomyces</i> sp.		<i>Forcepia</i> sp. (1999)
20	N203	<i>Streptomyces</i> sp.		<i>Forcepia</i> sp. (1999)
21	N212	<i>Streptomyces noboritoensis</i>		<i>Forcepia</i> sp. (1999)
22	N217	<i>Streptomyces</i> sp. S37		<i>Forcepia</i> sp. (1999)
23	N218	<i>Promicromonospora aerolata</i>		<i>Forcepia</i> sp. (1999)
24	N227	<i>Promicromonospora</i> <i>endophytica</i>		<i>Forcepia</i> sp. (1999)
25	N248	<i>Agrococcus casei</i>		<i>Forcepia</i> sp. (1999)
26	N816	<i>Streptomyces</i> sp.		<i>Scleritoderma cyanea</i> (2000)
27	P114	<i>Streptomyces</i> sp. FXJ1.412		<i>Discodermia</i> sp. (1996)
28	P183	<i>Streptomyces</i> sp. FiRh17		<i>Discodermia</i> sp. (1996)
29	P257	<i>Salinispora arenicola</i> CNY-685		<i>Discodermia</i> sp. (1996)
30	P419	<i>Streptomyces</i> sp. FZ92		<i>Discodermia</i> sp. (1998)

31	P901	<i>Pseudonocardia</i> sp. AL041005-10		<i>Discodermia</i> sp. (1998)
32	P947	<i>Mycobacterium</i> sp. L1906		<i>Discodermia</i> sp. (1998)
33	R529	<i>Nocardiopsis metallicus</i>	AY368531	<i>Gorgonacea</i> (2000)
34	R622	<i>Tsukamurella spongiae</i> K363		<i>Hexactinellida</i> + <i>Zoanthidea</i> (2000)
35	R783	<i>Pseudonocardia</i> sp.		<i>Forcepia</i> sp. (2001)
36	R786	<i>Streptomyces albidoflavus</i> S19		<i>Forcepia</i> sp. (2001)
37	R787	<i>Streptomyces ambofaciens</i> OZK18		<i>Forcepia</i> sp. (2001)
38	R817	<i>Streptomyces</i> sp. S15		<i>Forcepia</i> sp. (2001)
39	R818	<i>Streptomyces</i> sp.		<i>Forcepia</i> sp. (2001)
40	R858	<i>Pseudonocardia</i> sp.		<i>Forcepia</i> sp. (2001)
41	S181	<i>Pseudonocardia</i> sp.		<i>Discodermia</i> sp. (2000)
42	S355	<i>Streptomyces</i> sp.		<i>Forcepia</i> sp. (2001)
43	S579	<i>Pseudonocardia</i> sp. EC080619-01		<i>Forcepia</i> sp. (2001)
44	S653	<i>Actinomycetospora</i> sp.		<i>Forcepia</i> sp. (2001)
45	V324	<i>Streptomyces tendae</i>		<i>Theonellidae</i> n.sp. (2004)
46	V453	<i>Sphingomanas paucimobilis</i>		<i>Leiodermatium</i> sp. (2005)
47	V459	<i>Leifsonia</i> sp.		<i>Leiodermatium</i> sp. (2005)
48	V469	<i>Leifsonia</i> sp.		<i>Leiodermatium</i> sp. (2005)
49	V474	<i>Leifsonia</i> sp.		<i>Leiodermatium</i> sp. (2005)
50	X306	<i>Erythrobacter</i> sp.		<i>Leiodermatium</i> sp. (2010)

* Taxonomy is based on Blast analysis of 16S rDNA amplified from each strain.

** The year of sponge sample collection (Sfanos et al., 2005).

Table S2. Disk diffusion assay of extractions against *Candida albicans*; *Staphylococcus aureus* and MRSA, *Pseudomonas aeruginosa*. Inhibition is recorded as the diameter of the zone of growth inhibition (mm).

Strain	Inhibition zone (mm)				Concentration ⁴
	C.a.	MRSA	S.a.	P.a.	
E677	9 ¹	11	ND	ND	5
E677-la	10	10	ND	ND	5
J378	ND	12	9	ND	5
J378-la	ND	25	16	ND	5
J855	ND	ND	18	ND	5
J855-la	ND	ND	13	ND	5
L128	12 ¹	10	ND	ND	5
L128-la	ND	9 ¹	ND	ND	5
L733	ND	13	10	ND	5
L733-la	ND	12	10	ND	5
M864	ND	ND	30	ND	5
M864-la	ND	ND	32	ND	5
N197	ND	9 ¹	ND	ND	5
N197-la	ND	10 ¹	ND	ND	5
N200	ND	10	- ²	ND	10
N200-la	ND	10	- ²	ND	10
N201	10 ¹	16	- ²	9	10
N201-la	ND	13	- ²	ND	10
N203	12	18	- ²	14	10
N203-la	ND	11	- ²	ND	10
N217	11	20	14	14	5
N217-la	ND	16	13	10	5
N218	9	10 ¹	- ²	ND	10
N218-la	8 ¹	10 ¹	- ²	ND	10
N227	10 ¹	ND	- ²	ND	10
N227-la	12 ¹	ND	- ²	ND	10
N248	ND	ND	- ²	ND	10
N248-la	ND	10	- ²	ND	10

N816	ND	ND	ND	ND	5
N816-la	ND	11	12	ND ³	5
P114	ND	9	ND	ND	5
P114-la	13 ¹	10	ND	ND	5
P183	ND	9 ¹	ND	ND	5
P183-la	ND	9 ¹	ND	ND	5
P257	ND	ND	12	ND	5
P257-la	ND	ND	26	ND	5
P419	ND	8	ND	ND	5
P419-la	ND	ND	8	ND	5
P901	9	ND	ND	ND	5
P901-la	ND	ND	ND	ND	5
R786	22	12 ¹	ND	ND	5
R786-la	22	13 ¹	ND	ND	5
R787	ND	8	ND	ND	5
R787-la	ND	8	ND	ND	5
R817	ND	10	ND	ND	5
R817-la	ND	11 ¹	ND	ND	5
R818	ND	ND	- ²	ND	10
R818-la	21	ND	- ²	ND	10
S355	ND	ND	- ²	ND	10
S355-la	ND	10 ¹	- ²	ND	10
S653	ND	12	- ²	ND	10
S653-la	ND	9	- ²	ND	10
V324	ND	11	- ²	ND	5
V324-la	ND	15	- ²	ND	5
Positive control	27	19	22	23	

Note: C.a., *C. albicans*; S.a., *S. aureus*; P.a., *P. aeruginosa*. Positive controls are nystatin (100 U) for *C. albicans*; cefoxitin (30 µg) for MRSA; gentamicin (10 µg) for *S. aureus* and *P. aeruginosa*. ‘-la’, cultures supplemented with 2 mM LaCl₃; ND, activity not detected; ¹, the edge of inhibition zone is hazy; ⁻², not determined; ³, anti-P.a. activity was observed only using

partially purified fractions; ⁴, extractions are dissolved in methanol with a final concentration of 5 or 10 mg/mL.