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Supplementary Material

Trachycladindoles H-M: Molecular networking guided exploration of a library of southern Australian marine sponges.

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Figure S1. A portion of the GNPS molecular networks for 960 marine *n*-BuOH extracts and 95 authentic standards from different classes of marine natural compounds. Red box: trachycladindole clusters– see the expansion in **Figure S2.**

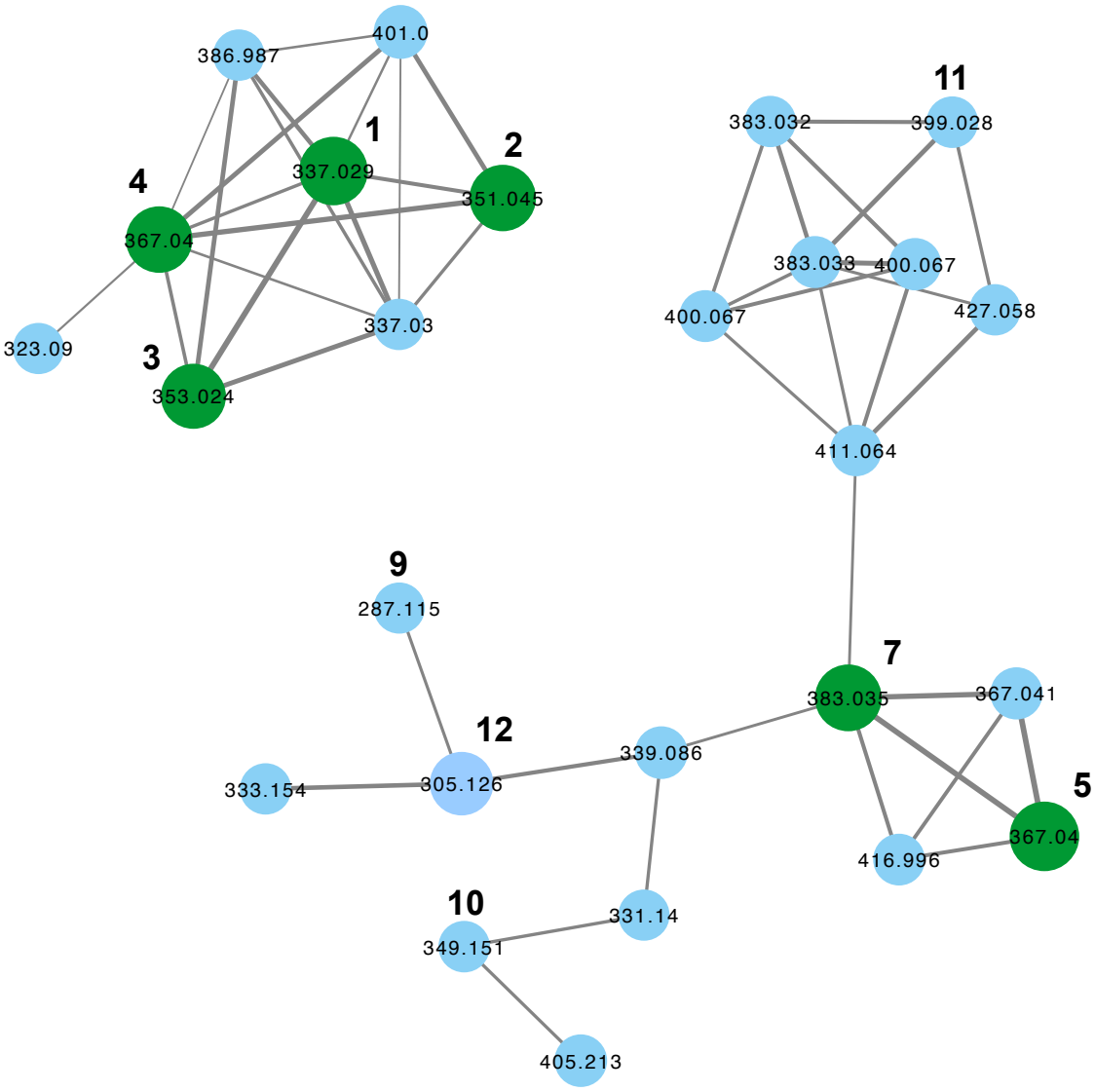
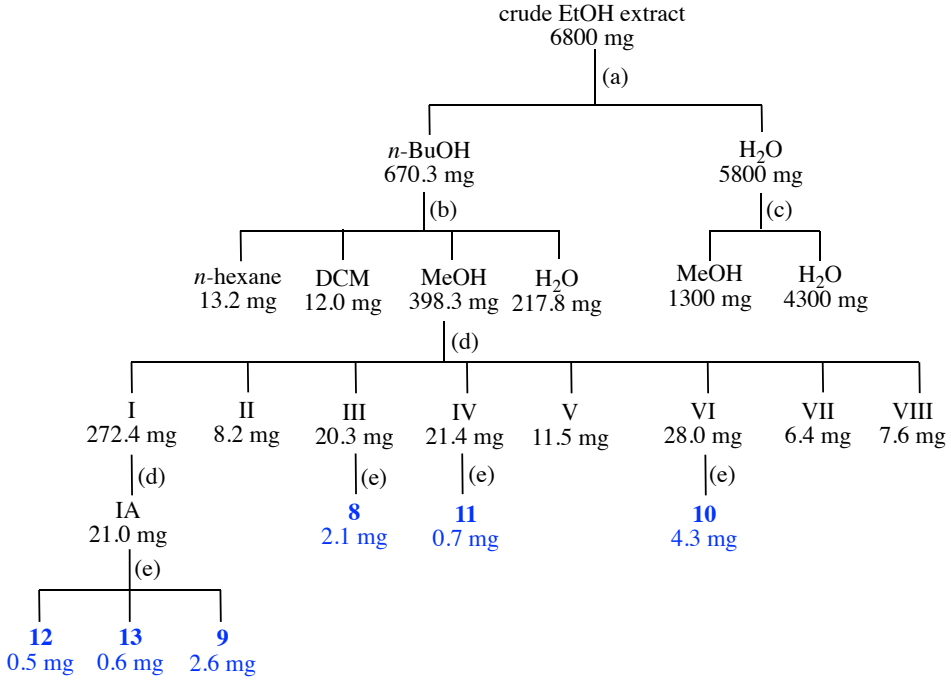


Figure S2. GNPS trachycladindole clusters inclusive of nodes attributed to (i) both authentic standards and CMB-01063 (green) and (ii) CMB-01063 only (blue); highlighting known trachycladindoles A–G (**1**–**5** and **7)**, and new trachycladindoles I–L (**9**–**12**)



(a) Partition [n-BuOH/H_2O]}
(b) Trituration [*n*-hexane, DCM, MeOH, H_2O]
(c) Trituration [MeOH and H_2O]
(d) Preparative HPLC (Agilent Zorbax SB-C_8 21.2 × 150 mm, 5 µm, 20 mL/min)
(e) Semi preparative HPLC (Agilent Zorbax SB-C_3 9.4 mm × 250 mm, 5 µm, 3 mL/min)

Scheme S1. Purification scheme for compounds **8-13**

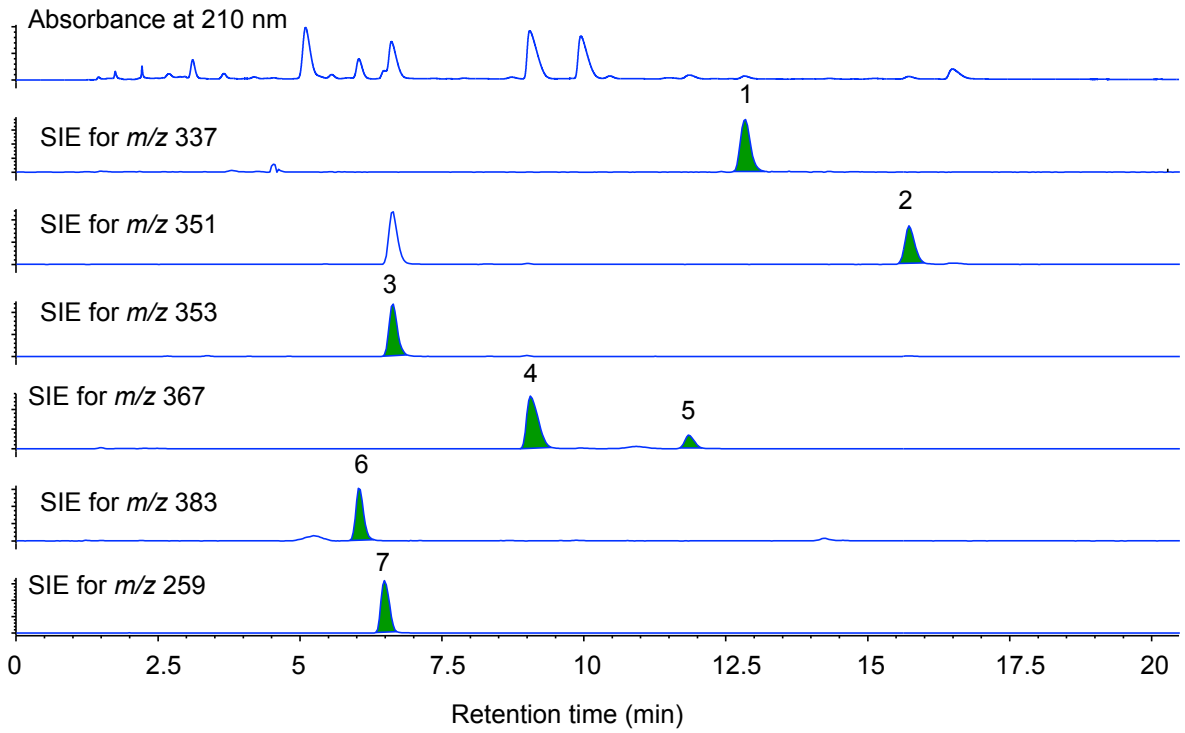


Figure S3. HPLC-DAD-MS (210 nm) chromatogram (Zorbax C_3 column) of CMB-01063 BuOH/MeOH extract, with single ion extraction (SIE) and comparison with authentic standard showed the presence of trachycladindoles A–G (**1–7**)

Table S1. NMR (600 MHz, methanol-d_4) data for trachycladindole H (**8**)

no.	δ_C	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H		11.73, br s		
2	128.0			
2-CO_2H	163.2			
3	106.6			
3a	124.4			
4	124.9	7.64, s		5, 7a, 6, 7, 3
5	108.4			
6	154.1			
7	98.5	7.03, s		5, 3a, 6, 7a
7a	138.0			
8	122.4			
9	117.0	6.97, s		8, 11, 3
N10- CH_3	33.0	3.61, s		9, 11
11	148.2			
N12- CH_3	30.8	3.26, s		8, 11

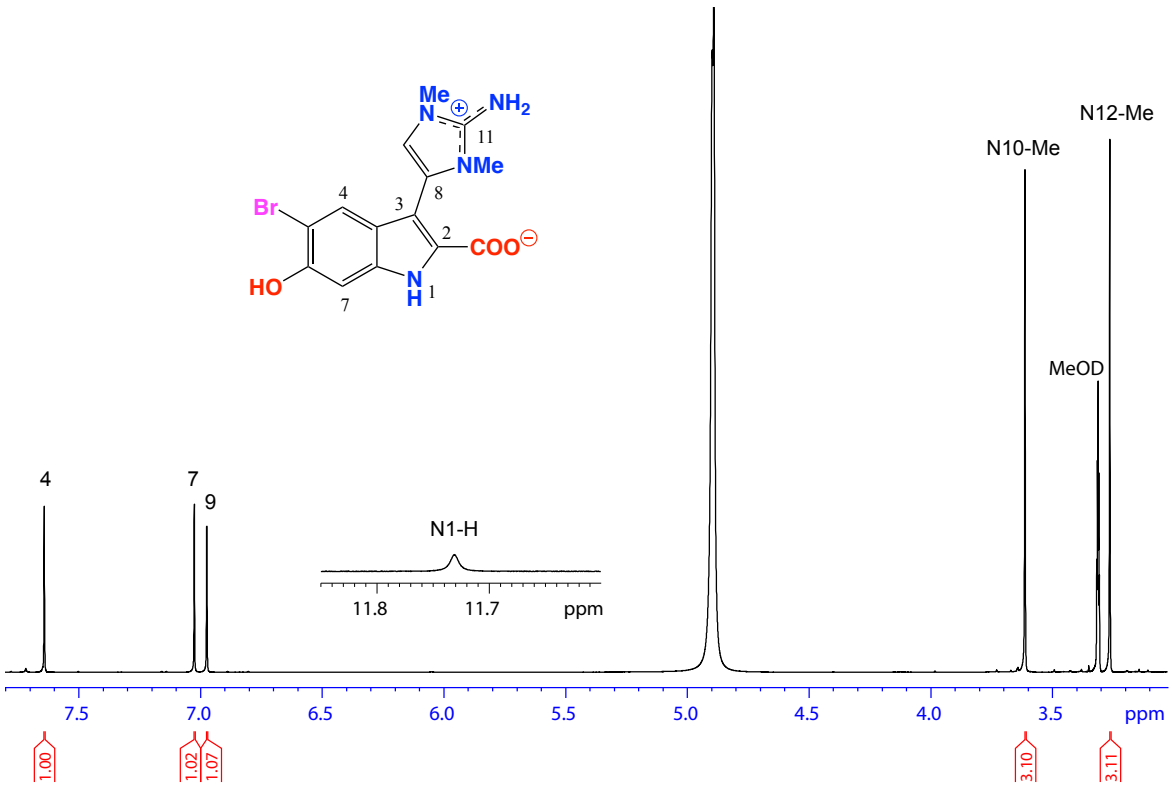


Figure S4. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole H (**8**)

Table S2. NMR (600 MHz, methanol-d_4) data for trachycladindole I (**9**)

no.	δ_C	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H				
2	127.2			
2-CO_2H	163.7			
3	107.3			
3a	123.0			
4	121.8	7.32, d (8.8)	5	7a, 6, 7, 3
5	114.3	6.76, dd (8.8, 2.1)	4, 7	7, 3a
6	158.0			
7	97.5	6.87, d (2.1)	5	5, 3a, 6
7a	139.1			
8	123.4			
9	116.8	6.94, s		8, 11, N10- $\underline{C}H_3$, 3
N10-CH_3	33.0	3.61, s		9, 11
11	148.0			
N12-CH_3	30.8	3.27, s		8, 11

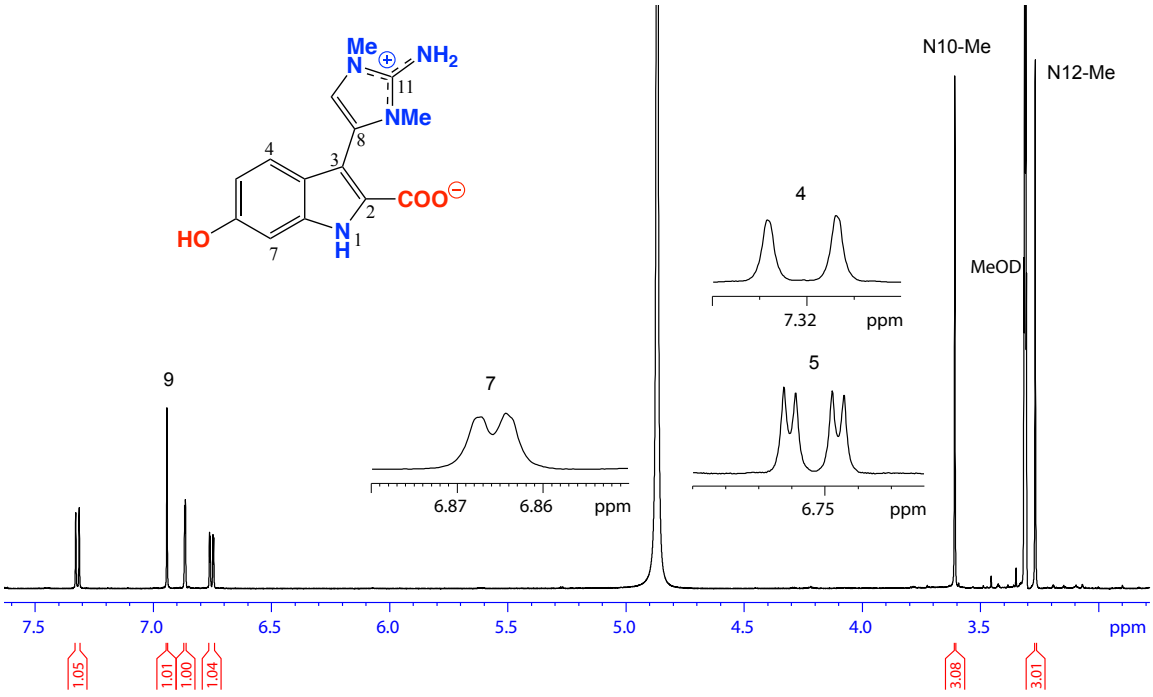


Figure S5. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole I (**9**)

Table S3. NMR (600 MHz, methanol-d_4) data for trachycladindole J (**10**)

no.	δ_C	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H				
2	130.5			
2-CO_2H	163.0			
3	106.1			
3a	131.1			
4	123.5	7.69, s	6	7a, 5, 6, 7, 3
5	115.8			
6	129.8	7.46[a], m		7a, 4
7	115.5	7.47[a], m	7	5, 3a
7a	136.2			
8	122.0			
9	117.3	6.99, s		8, 11, 3
N10-CH_3	33.1	3.62, s		9, 11
11	148.2			
N12-CH_3	30.8	3.26, s		8, 11

[a] overlapping resonances

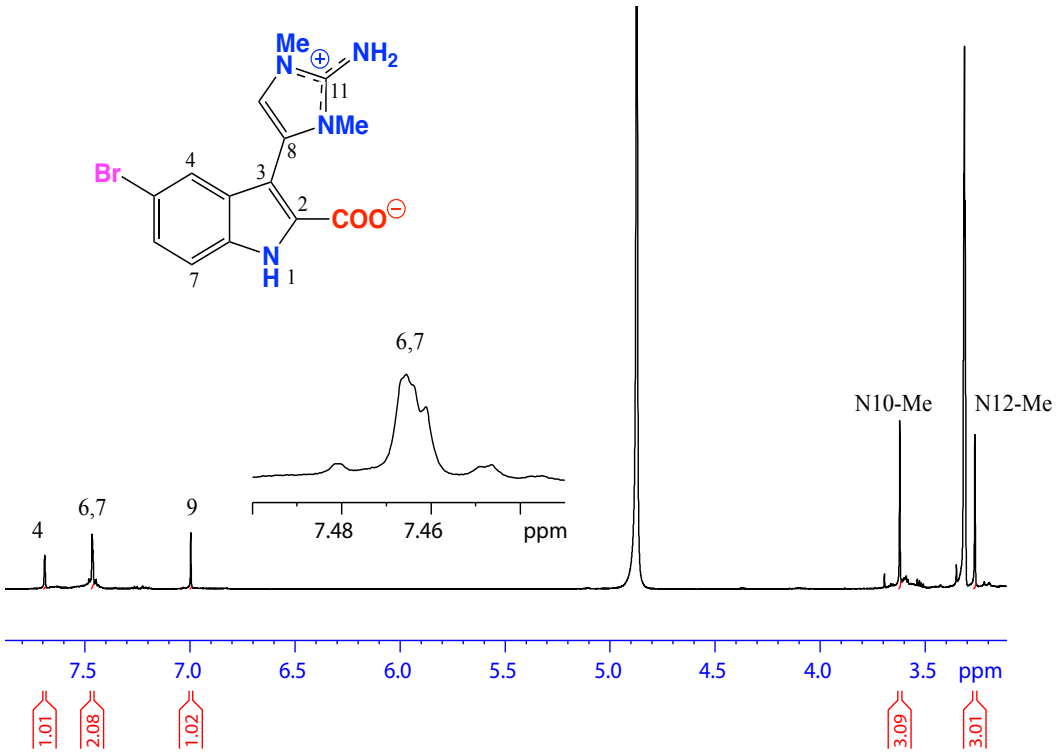


Figure S6. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole J (**10**)

Table S4. NMR (600 MHz, methanol-d_4) data for trachycladindole K (**11**)

no.	δ_C[a]	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H				
2	n.d.[b]			
2-CO_2H	162.8			
3	108.5			
3a	n.d.			
4	123.0	7.56, s		3, 6, 7a
5	n.d.			
6	149.4			
7	n.d.			
7a	135.1			
8	121.5			
9	117.0	6.89, s		8, 11
N10- CH_3	32.8	3.58, s		9, 11
11	147.7			
N12- CH_3	30.5	3.20, s		8, 11

[a] Carbon chemical shifts were determined from HSQC and HMBC correlations. [b] Resonances were not detected in the spectra.

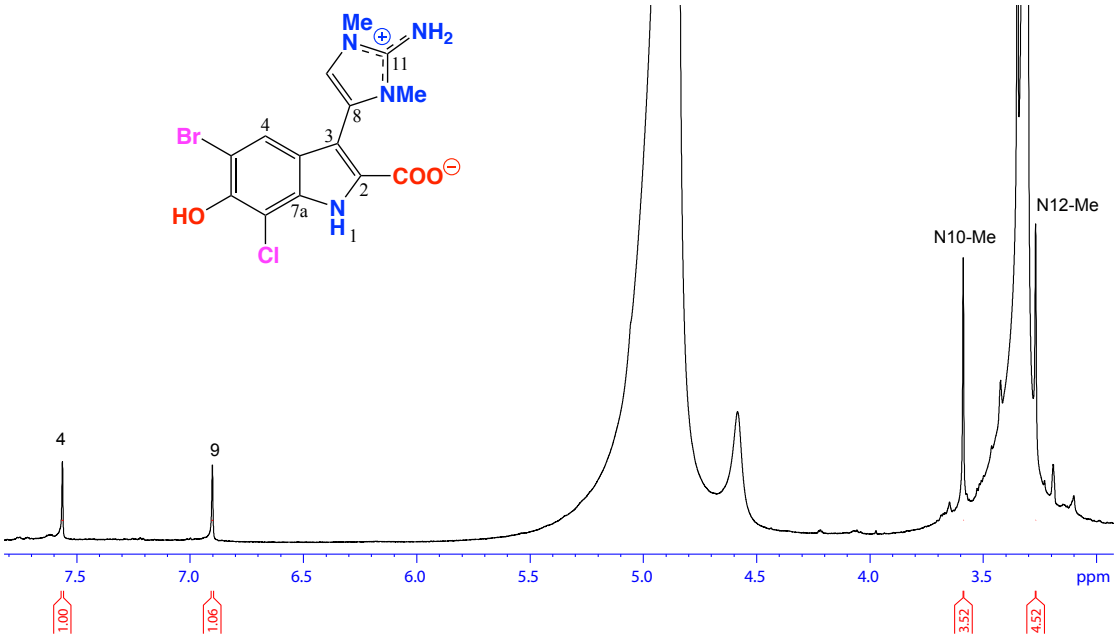


Figure S7. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole K (**11**)

Table S5. NMR (600 MHz, methanol-d_4) data for trachycladindole L (**12**)

no.	δ_C[a]	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H				
2	n.d.[b]			
2-CO_2H	168.7			
3	n.d.			
3a	120.4			
4	120.6	7.02, br s		
5	112.3	6.65, d (8.5)	7	3a, 6, 7
6	156.3			
7	98.1	6.84, s	5	5, 6, 3a, 7a
7a	138.2			
8	65.7	5.30, br s		
9	89.8	6.10, br s		
N10- CH_3	29.4	3.12, s		9, 11
11	157.9			
N12- CH_3	30.0	2.79, s		8, 11

[a] Carbon chemical shifts were determined from HSQC and HMBC correlations. [b] Resonances were not detected in the spectra.

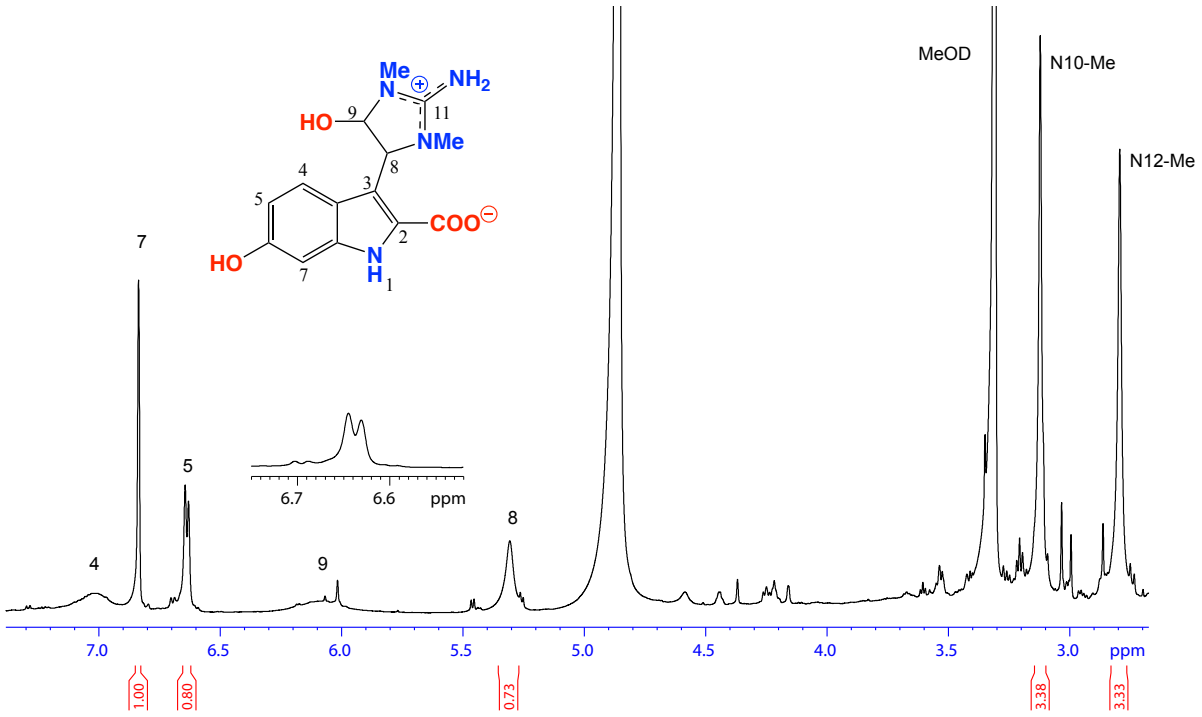


Figure S8. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole L (**12**)

Table S6. NMR (600 MHz, methanol-d_4) data for trachycladindole M (**13**)

no.	δ_C[a]	δ_H, mult. (*J* in Hz)	COSY	HMBC
N1-H				
2	n.d[b]			
2-CO_2H	n.d			
3	n.d			
3a	120.0			
4	121.0	7.29, br d (8.7)	5	6
5	112.1	6.66, d (8.7)	4, 7	3a, 7
6	156.1			
7	97.9	6.84, s	5	3a, 5, 6
7a	n.d			
8	58.9	6.36, br s		
9	48.5	a 4.04, dd (10.1, 10.1)	9b	
		b 3.73, m	9a	
N10-H				
11	160.0			
N12-CH_3	29.4	2.73, s		8, 11

[a] Carbon chemical shifts were determined from HSQC and HMBC correlations. [b] Resonances were not detected in the spectra.

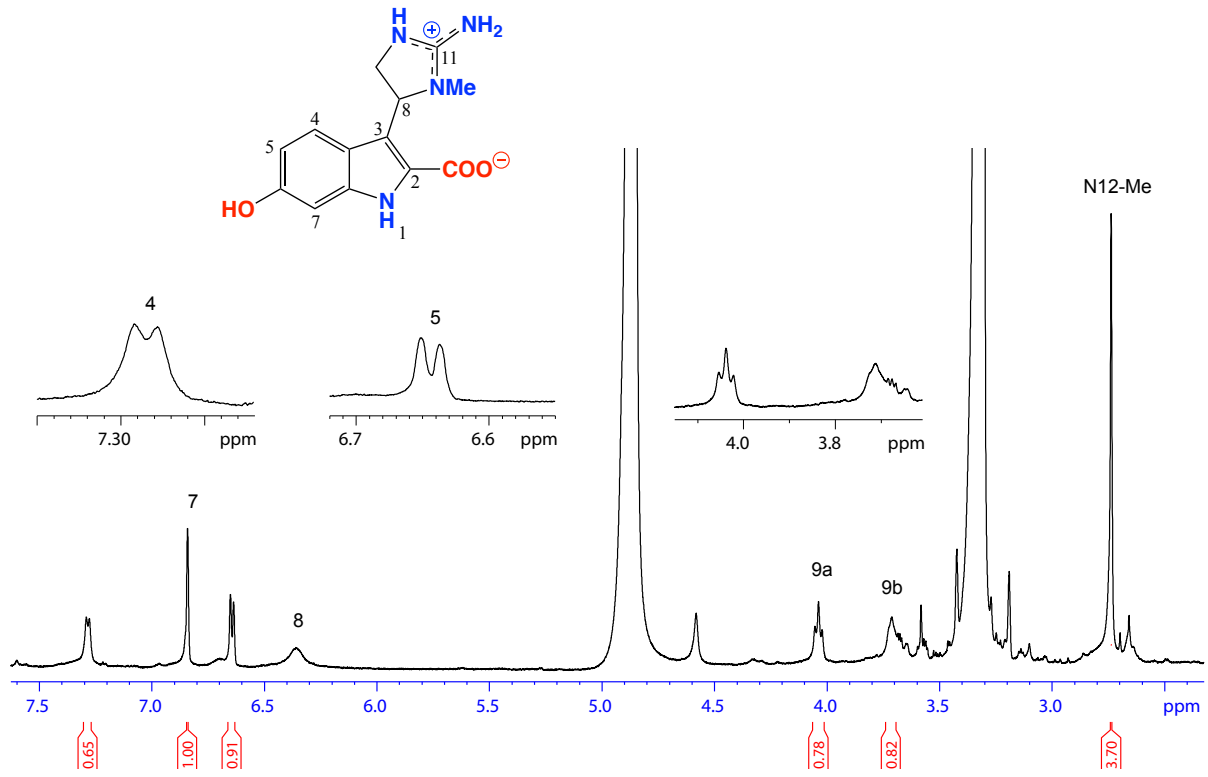


Figure S9. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of trachycladindole M (**13**)

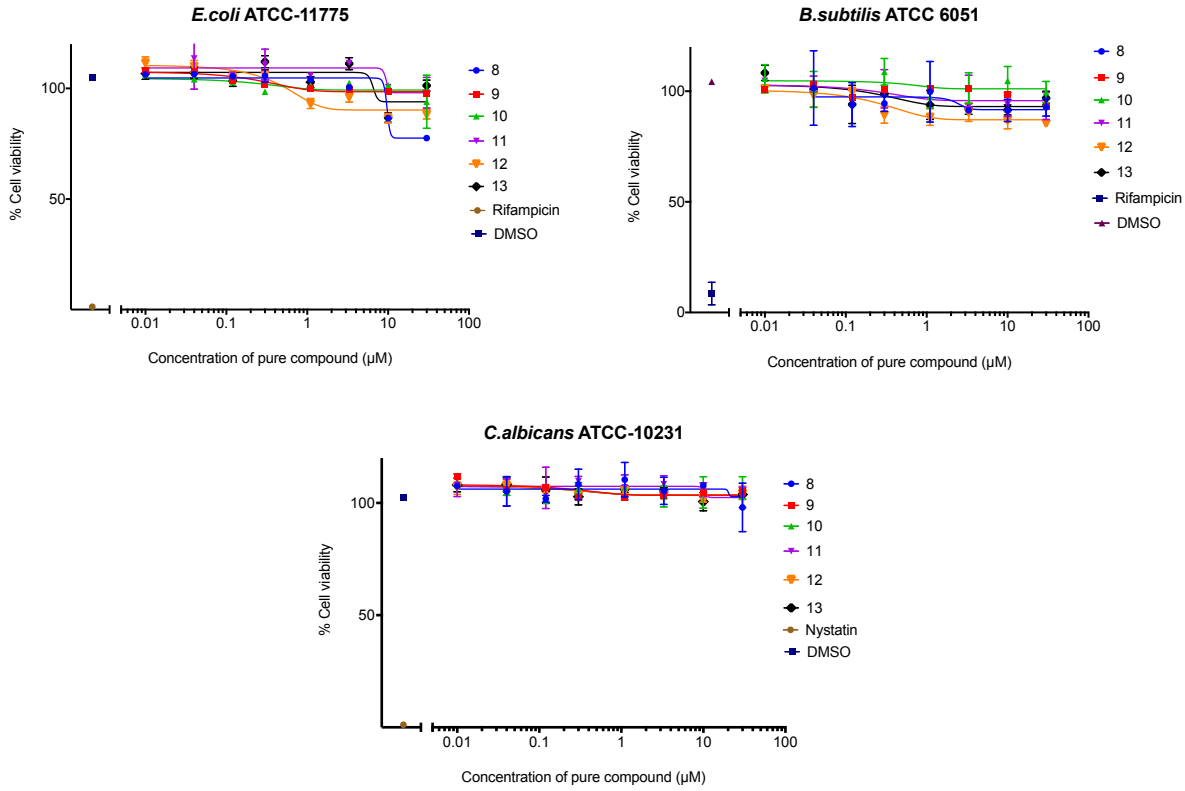


Figure S10. Antimicrobial activity of trachycladindoles (**8-13**)

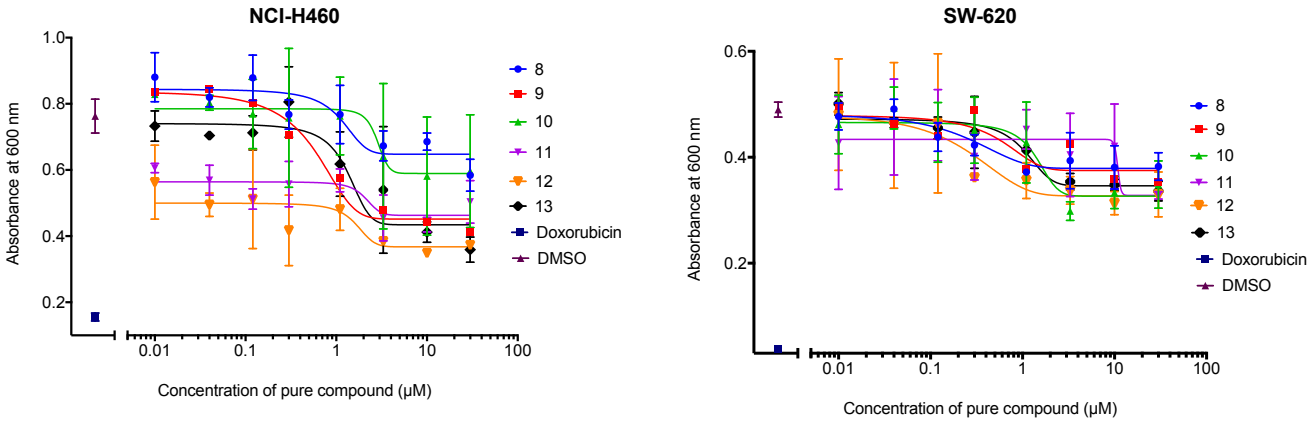


Figure S11. Cytotoxicity of trachycladindoles (**8-13**) against 2 cell lines