Supporting information

A Comprehensive Apoptotic Assessment of Niloticin in Cervical Cancer Cells: A tirucallane-type triterpenoid from Aphanamixis polystachya (Wall.) Parker

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1. **Cytotoxic studies of *A. polystachya* acetone extract in HeLa cell**

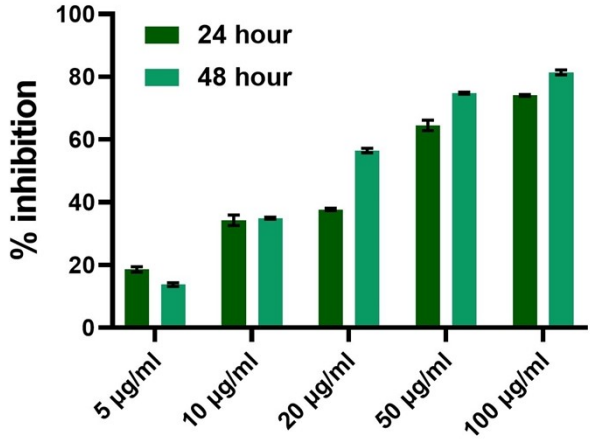


Figure S1. Assessment of cytotoxicity of acetone extracts at 24 hour and 48 hours in HeLa cells

% inhibition		
Conc. (µg/ml)	24 hour	48 hour
5	18.03	13.36
10	35.48	34.71
20	37.47	55.99
50	63.40	74.54
100	73.90	80.86

Table S1. Percentage inhibition of acetone extracts at 24 hour and 48 hours in HeLa cells

2. **Schematic representation of extraction and isolation procedure**

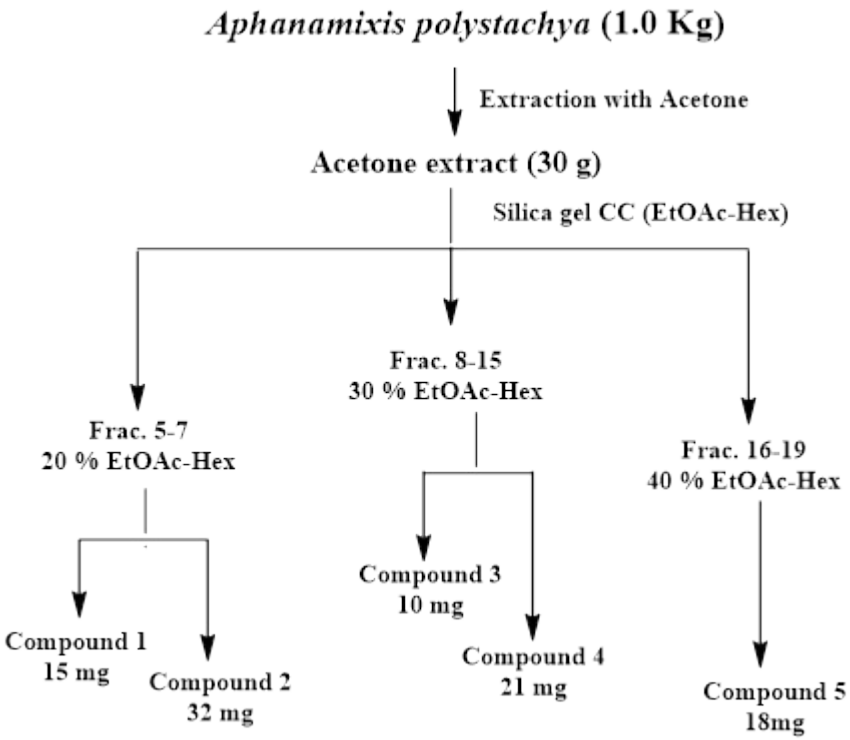


Figure S2. Extraction diagram and isolation procedure

3. Characterization of isolated molecules

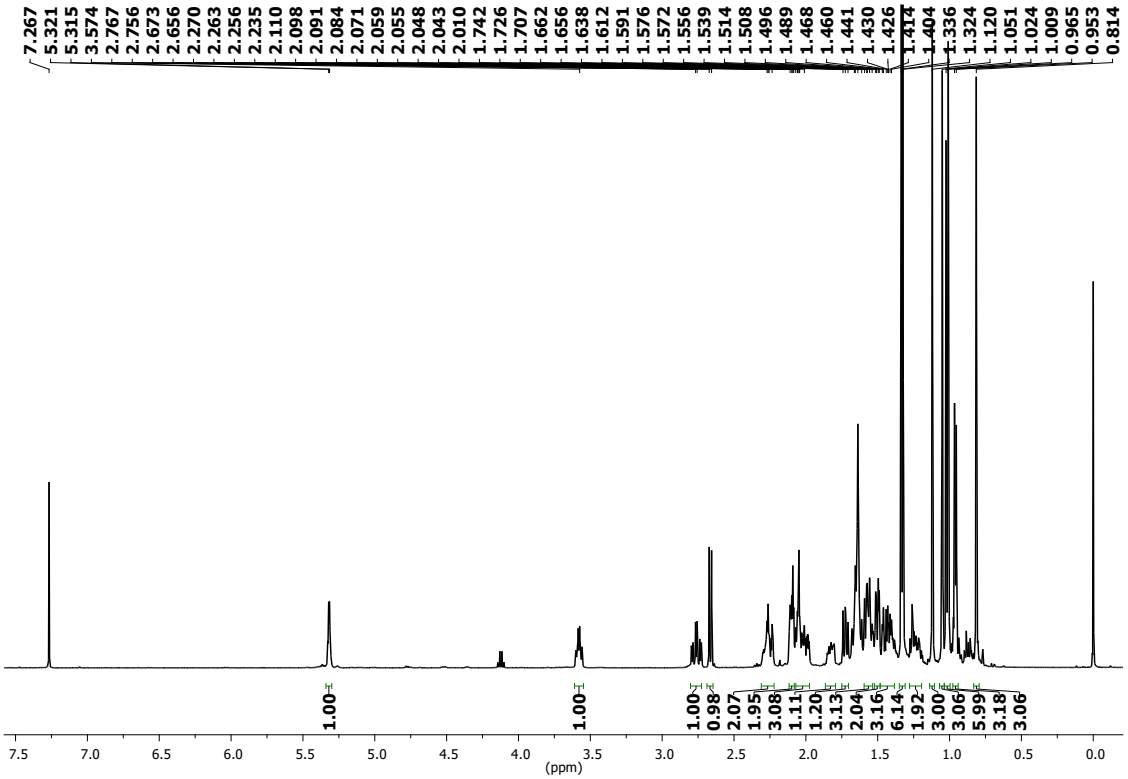


Figure S3. ^{1}H NMR spectrum of Niloticin (1) in $CDCl_3$

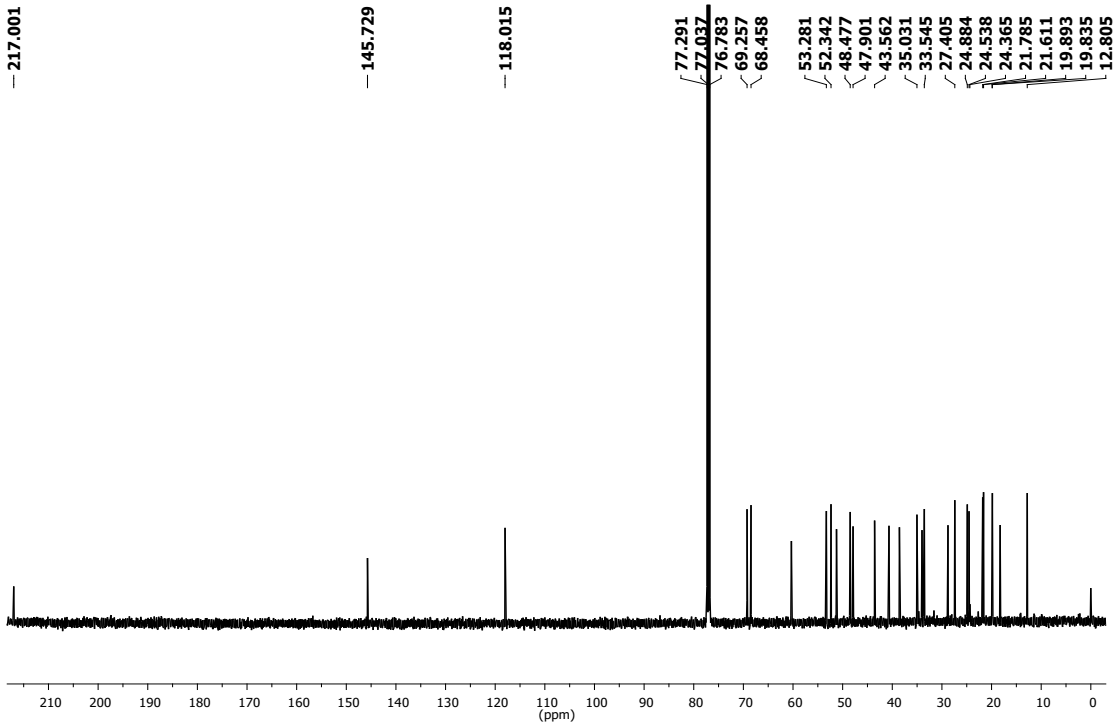


Figure S4. ^{13}C NMR spectrum of Niloticin (1) in $CDCl_3$

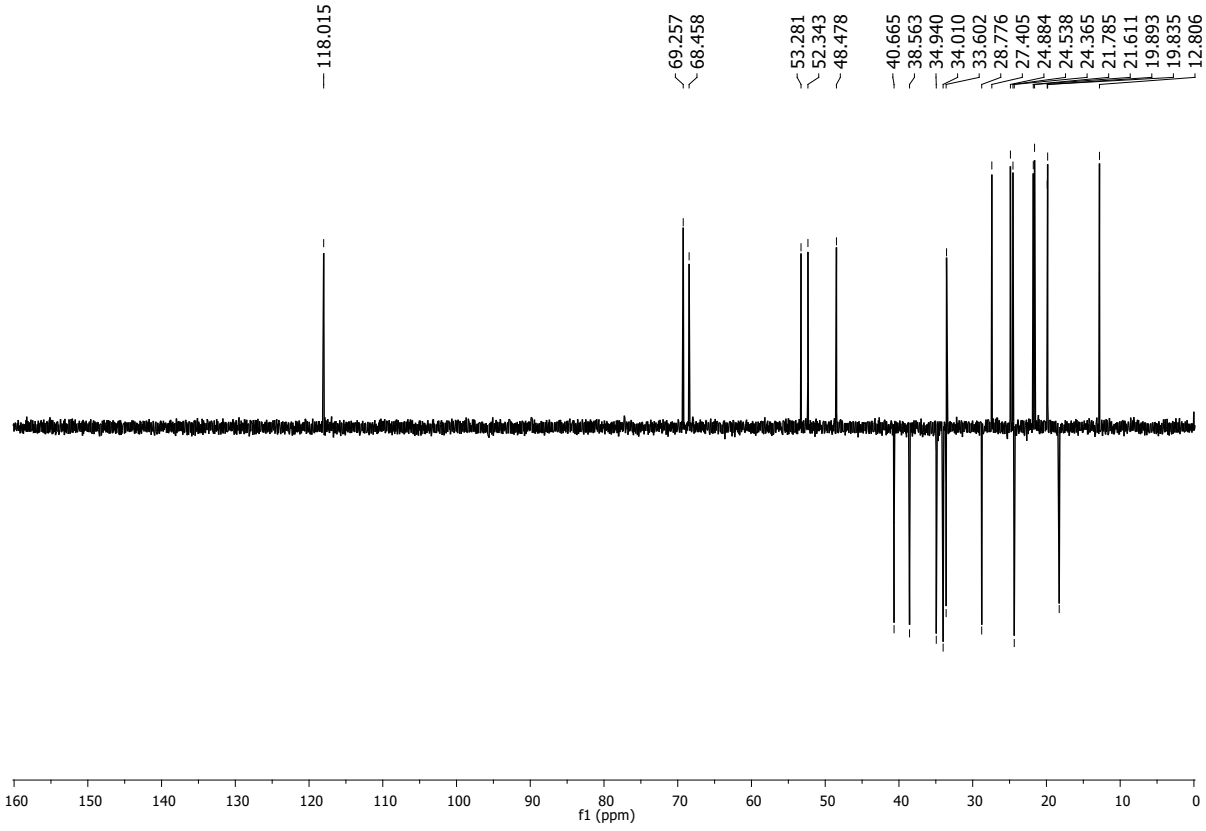


Figure S5. DEPT 135 NMR spectrum of Niloticin (1) in $CDCl_3$

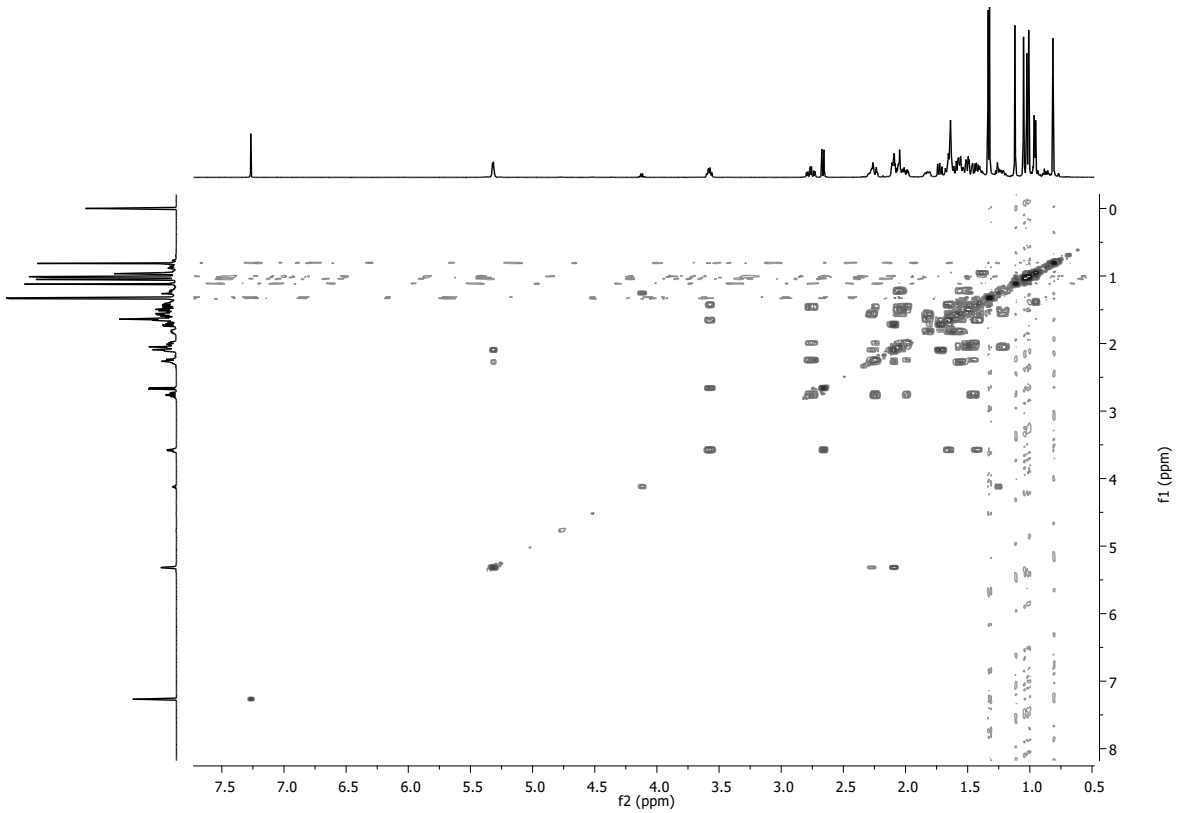


Figure S6. ^{1}H-^{1}H COSY NMR spectrum of Niloticin (1) in $CDCl_3$

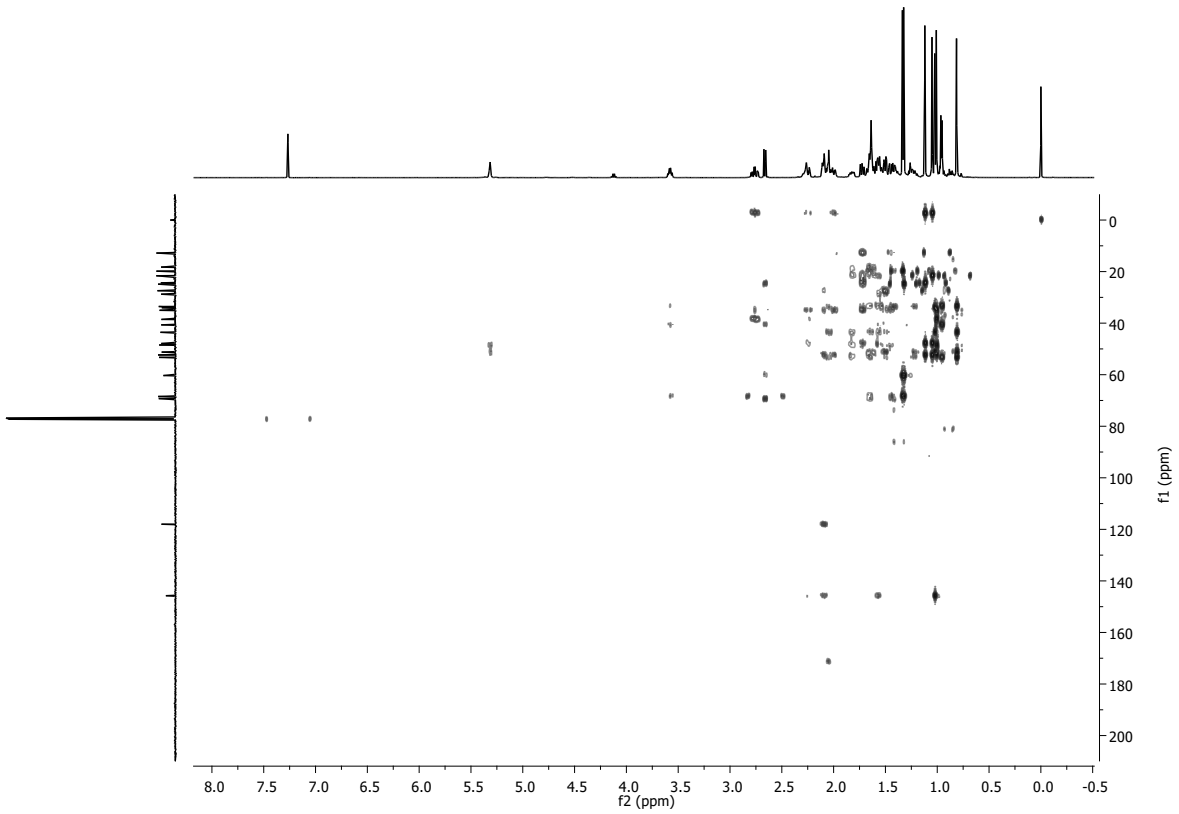


Figure S7. HMBC spectrum of Niloticin (1) in $CDCl_3$

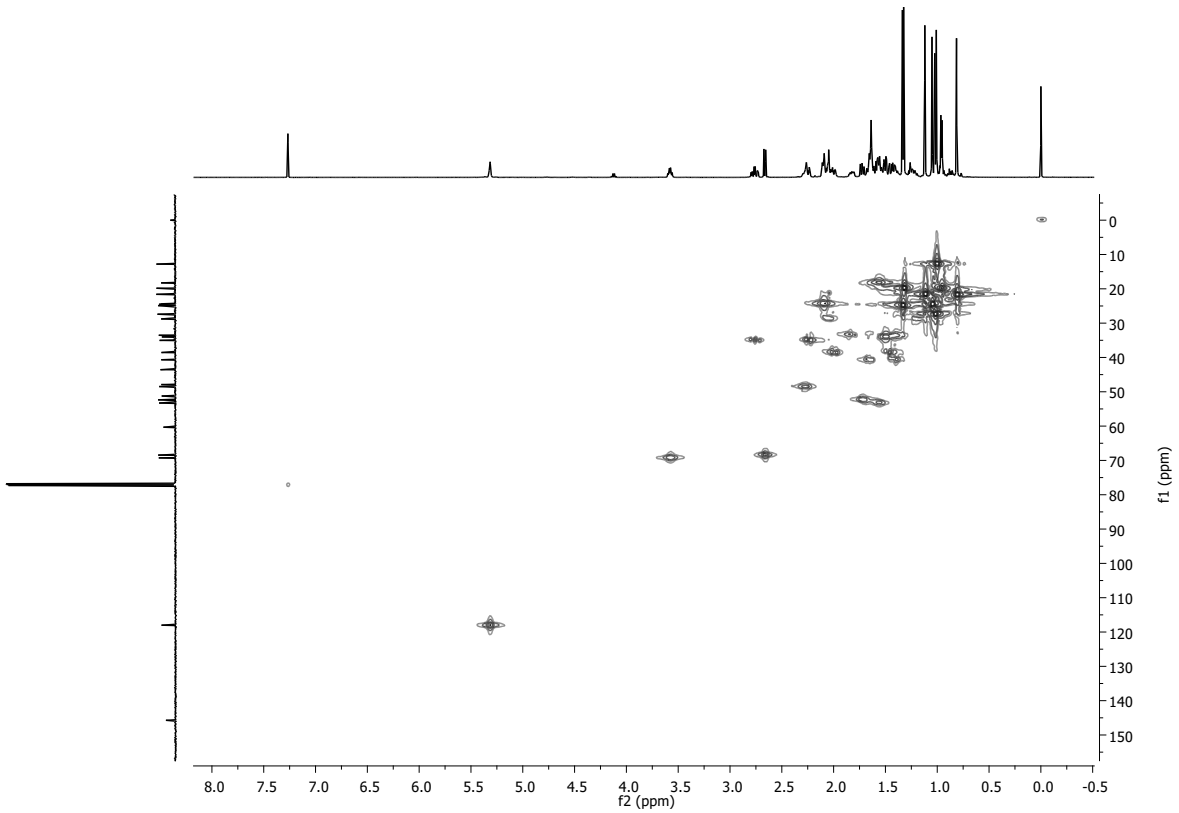


Figure S8. HMQC spectrum of Niloticin (1) in $CDCl_3$

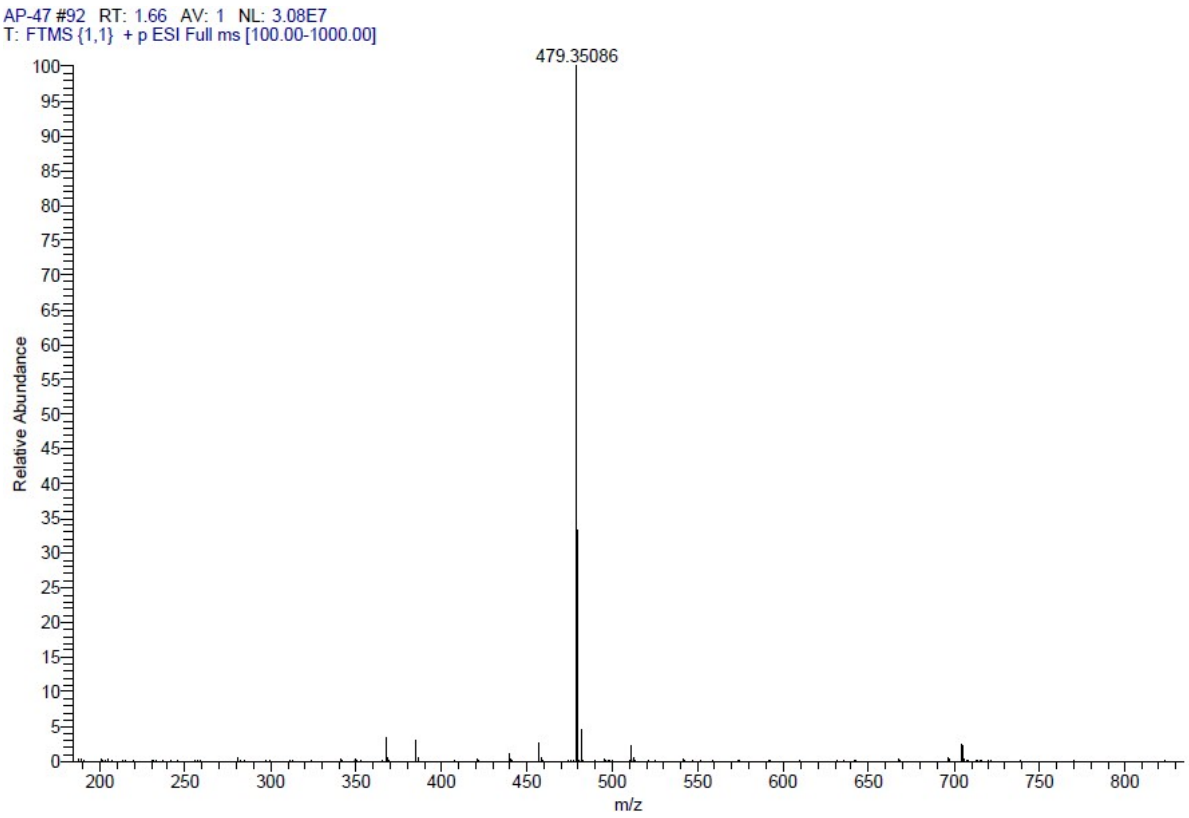
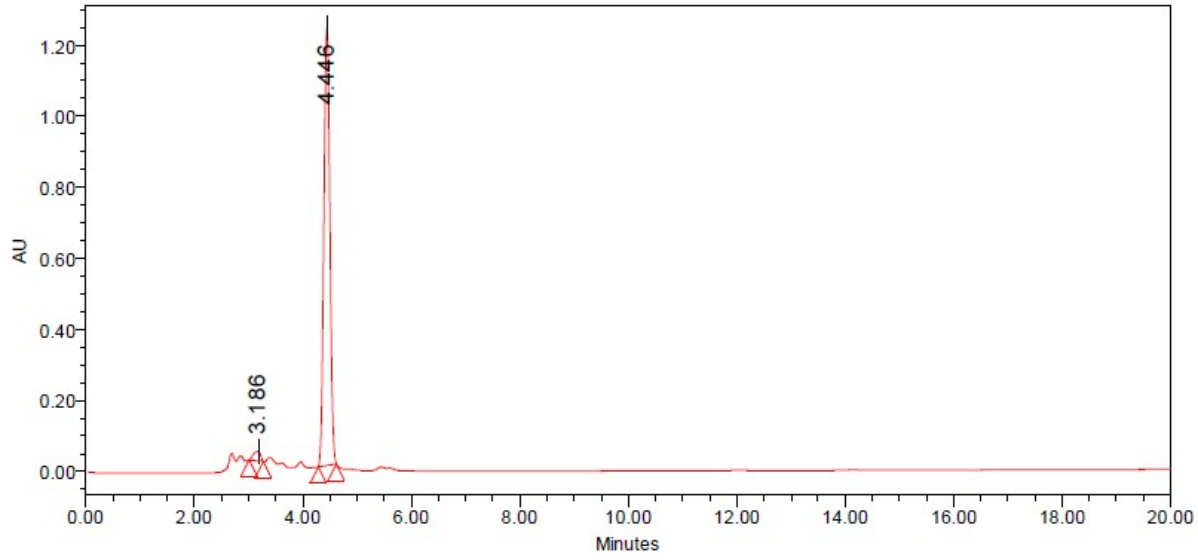


Figure S9: HRMS spectrum of Niloticin



Processed Channel: 2998 PDA 209.6 nm (2998 (210-400)nm)

	Processed Channel	Retention Time (min)	Area	% Area	Height
1	2998 PDA 209.6 nm (2998 (210-400)nm)	3.186	244113	2.52	27438
2	2998 PDA 209.6 nm (2998 (210-400)nm)	4.446	9437681	97.48	1232351

Figure S10. HPLC chromatogram of niloticin

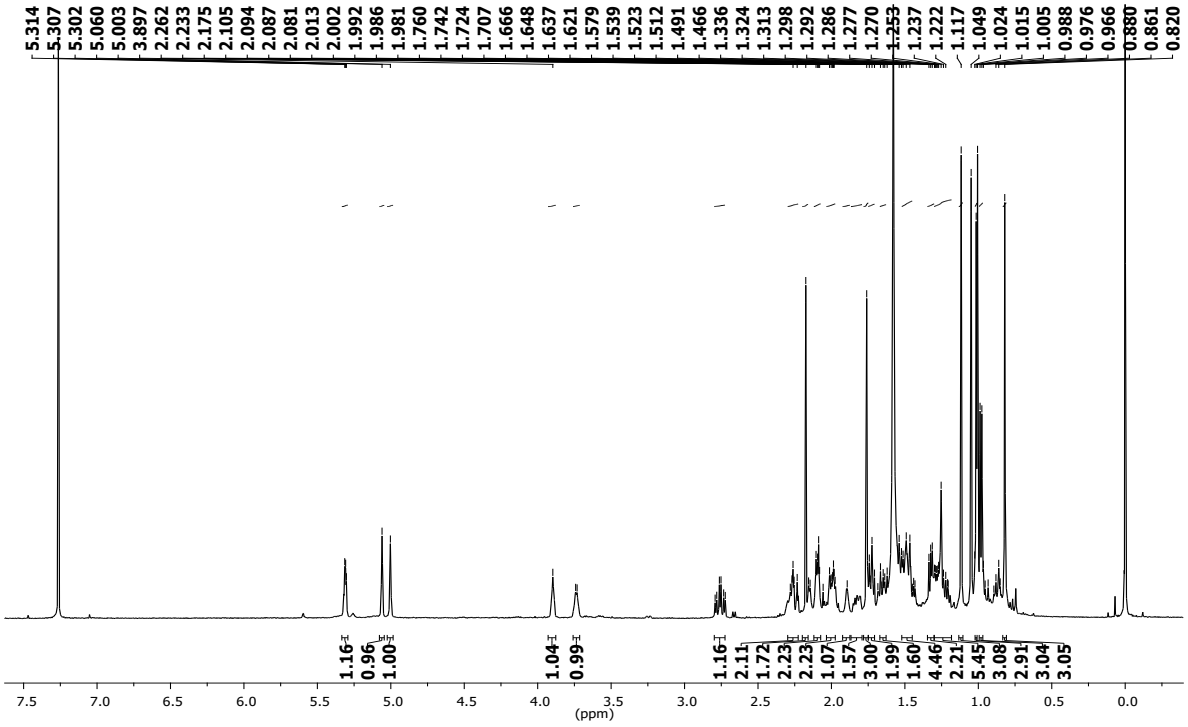


Figure S11. ^{1}H NMR spectrum of Bourjotinolone B (2) in $CDCl_3$

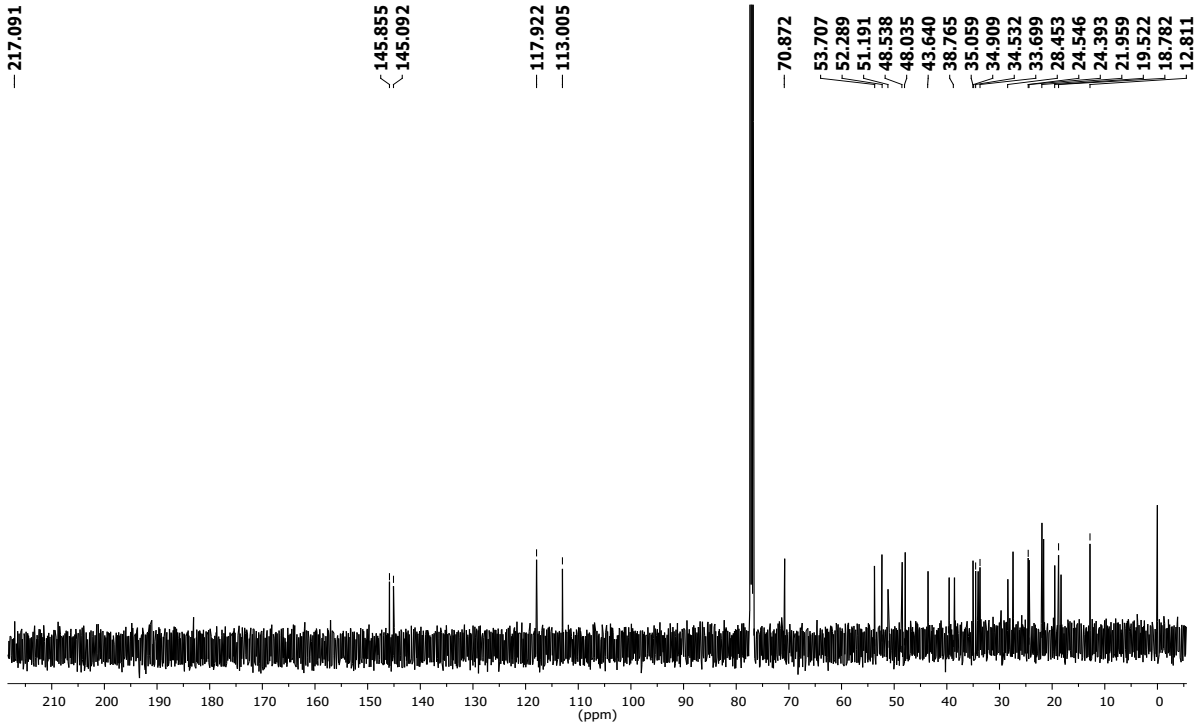


Figure S12. ^{13}C NMR spectrum of Bourjotinolone B (2) in $CDCl_3$

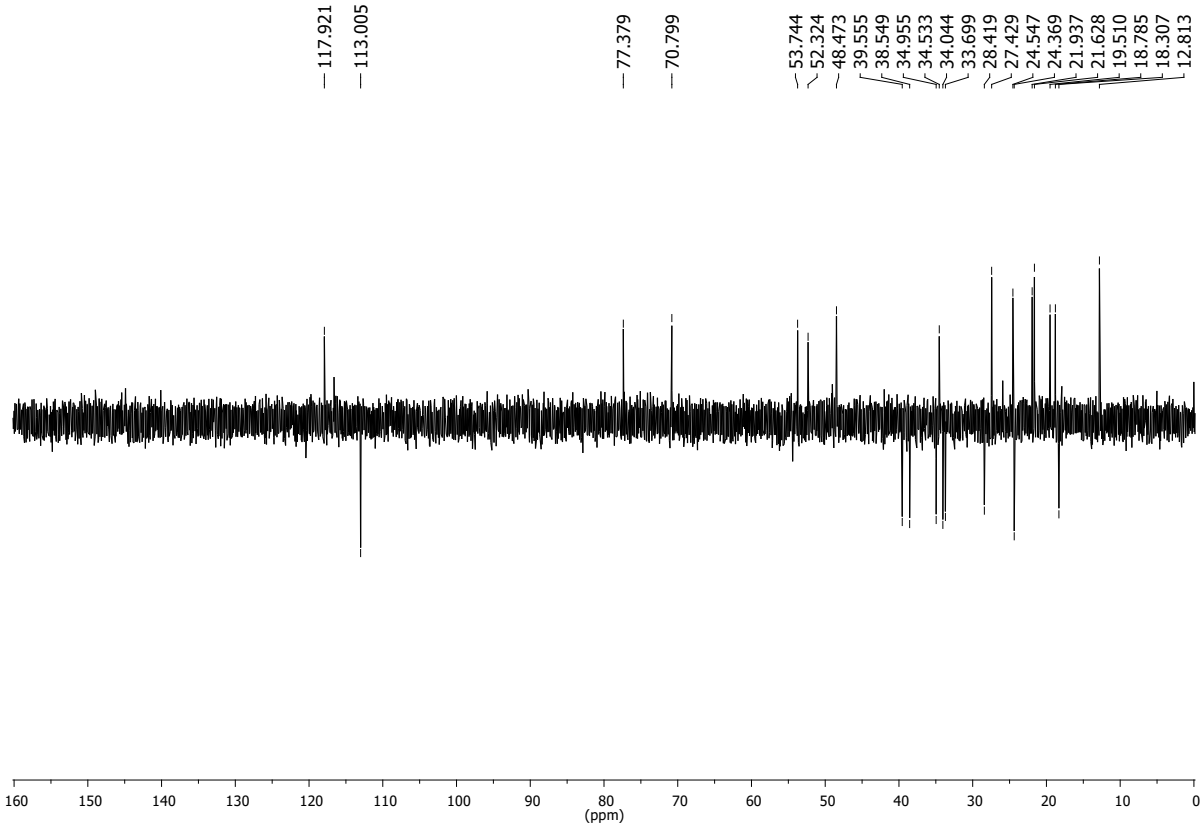


Figure S13. DEPT 135 NMR spectrum of Bourjotinolone B (2) in $CDCl_3$

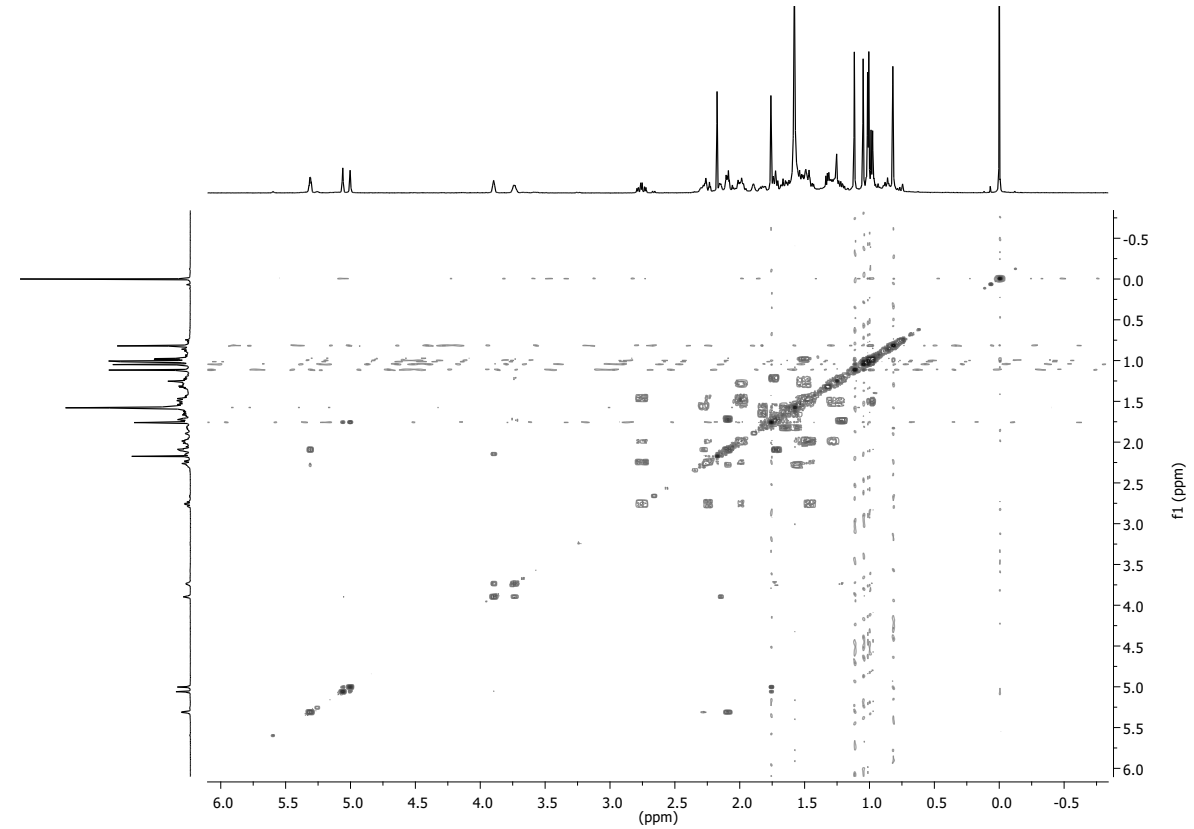


Figure S14. ^{1}H-^{1}H COSY NMR spectrum of Bourjotinolone B (2) in $CDCl_3$

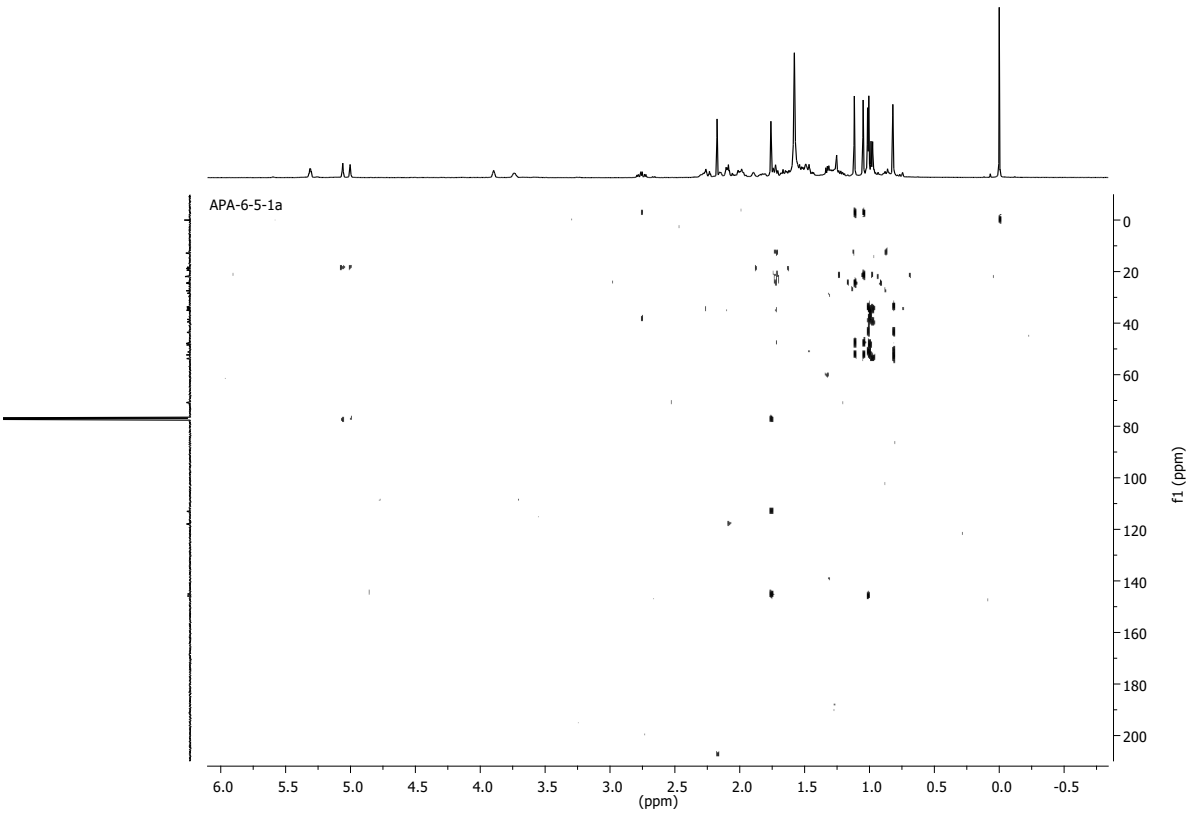


Figure S15. HMBC spectrum of Bourjotinolone B (2) in $CDCl_3$

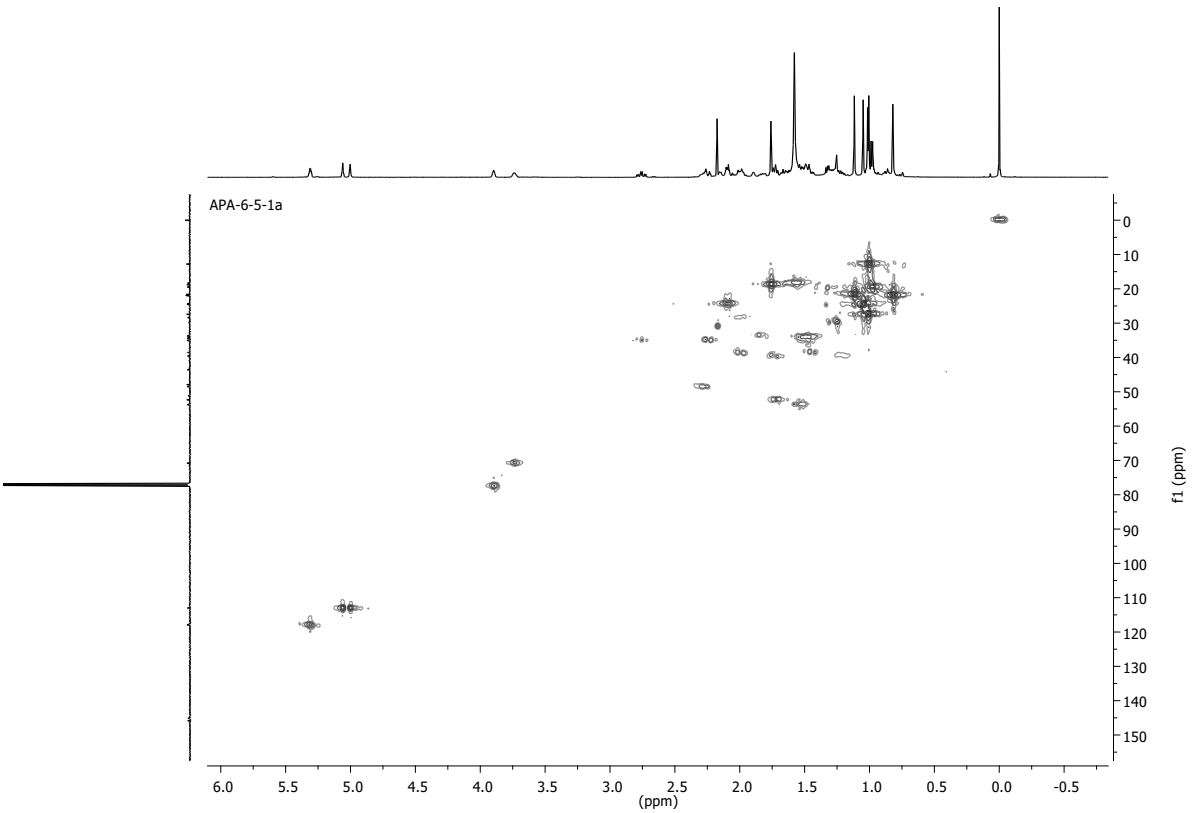


Figure S16. HMQC spectrum of Bourjotinolone B (2) in $CDCl_3$

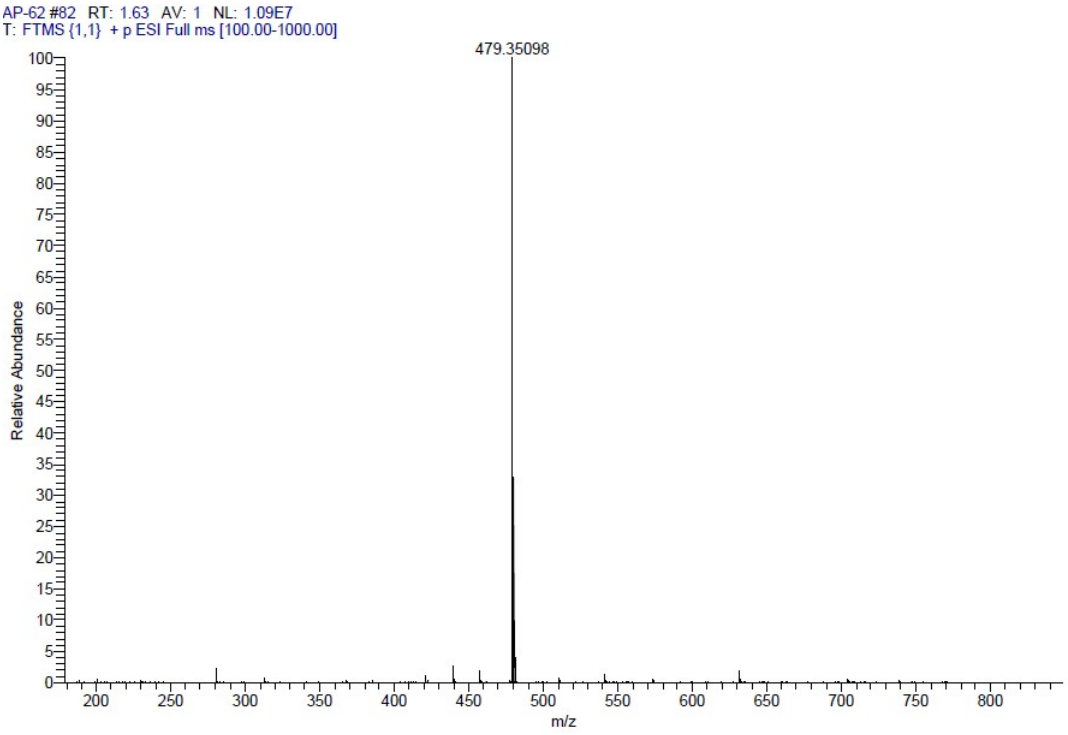
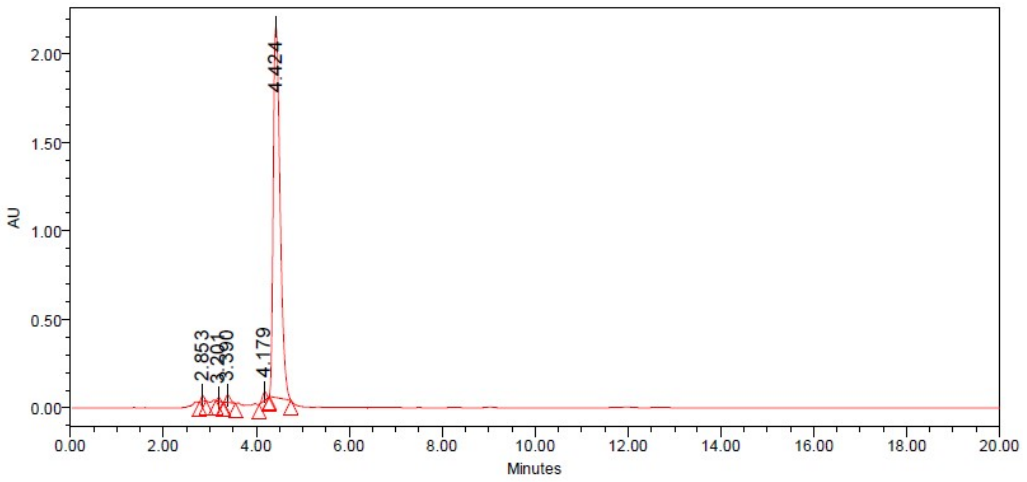


Figure S17: HRMS spectrum of Bourjotinolone B



Processed Channel: 2998 PDA 209.6 nm (2998 (210-400)nm)

	Processed Channel	Retention Time (min)	Area	% Area	Height
1	2998 PDA 209.6 nm (2998 (210-400)nm)	2.853	144345	0.65	33371
2	2998 PDA 209.6 nm (2998 (210-400)nm)	3.201	90926	0.41	20389
3	2998 PDA 209.6 nm (2998 (210-400)nm)	3.390	292590	1.32	42458
4	2998 PDA 209.6 nm (2998 (210-400)nm)	4.179	293511	1.32	48408
5	2998 PDA 209.6 nm (2998 (210-400)nm)	4.424	21332992	96.29	2096888

Figure S18. HPLC chromatogram of Bourjotinolone B

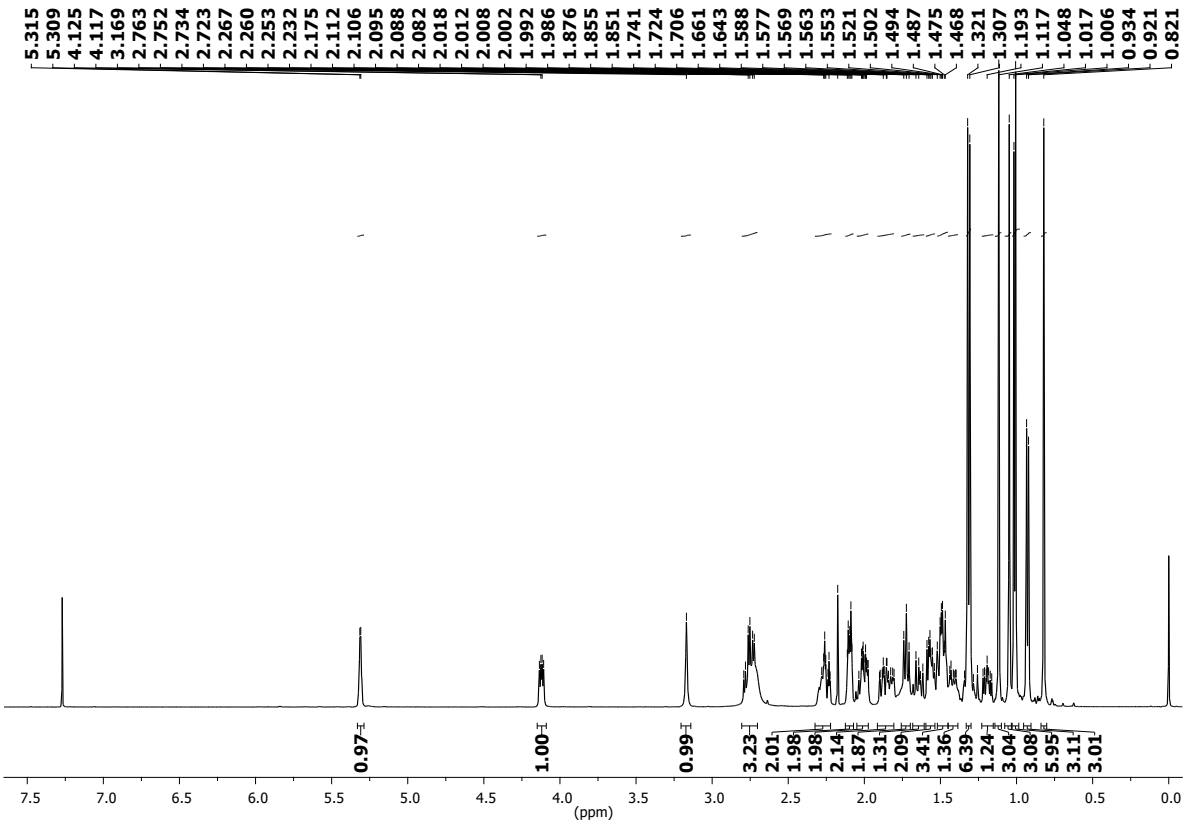


Figure S19. ^{1}H NMR spectrum of Piscidinol A (3) in $CDCl_3$

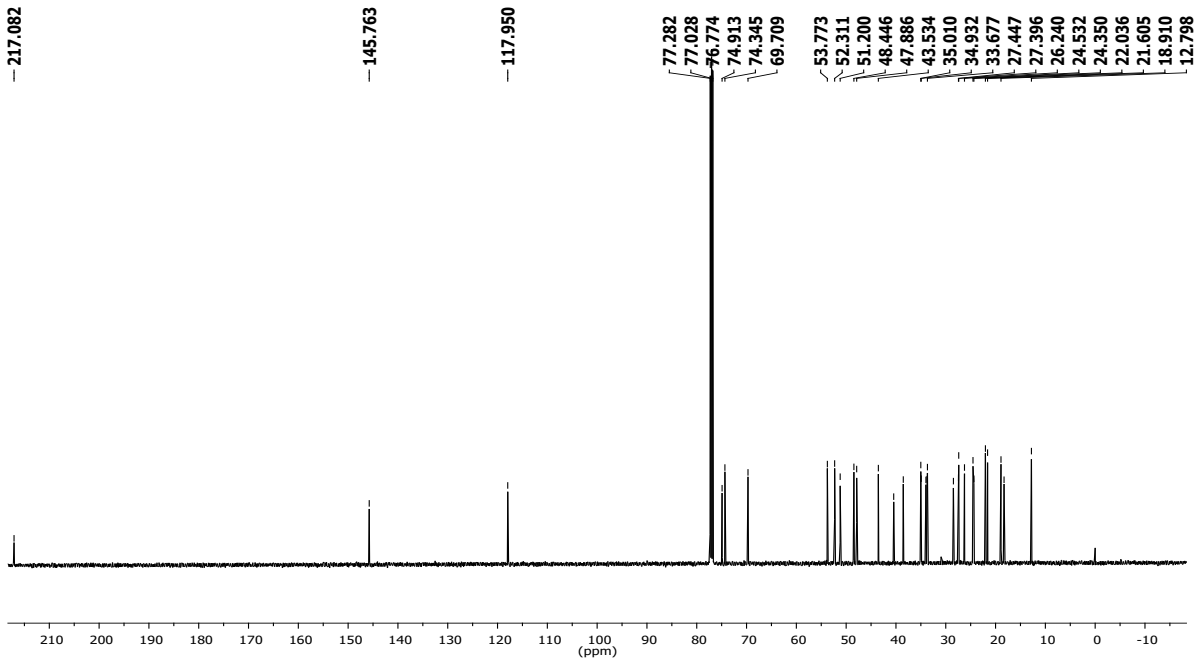


Figure S20. ^{13}C NMR spectrum of Piscidinol A (3) in $CDCl_3$

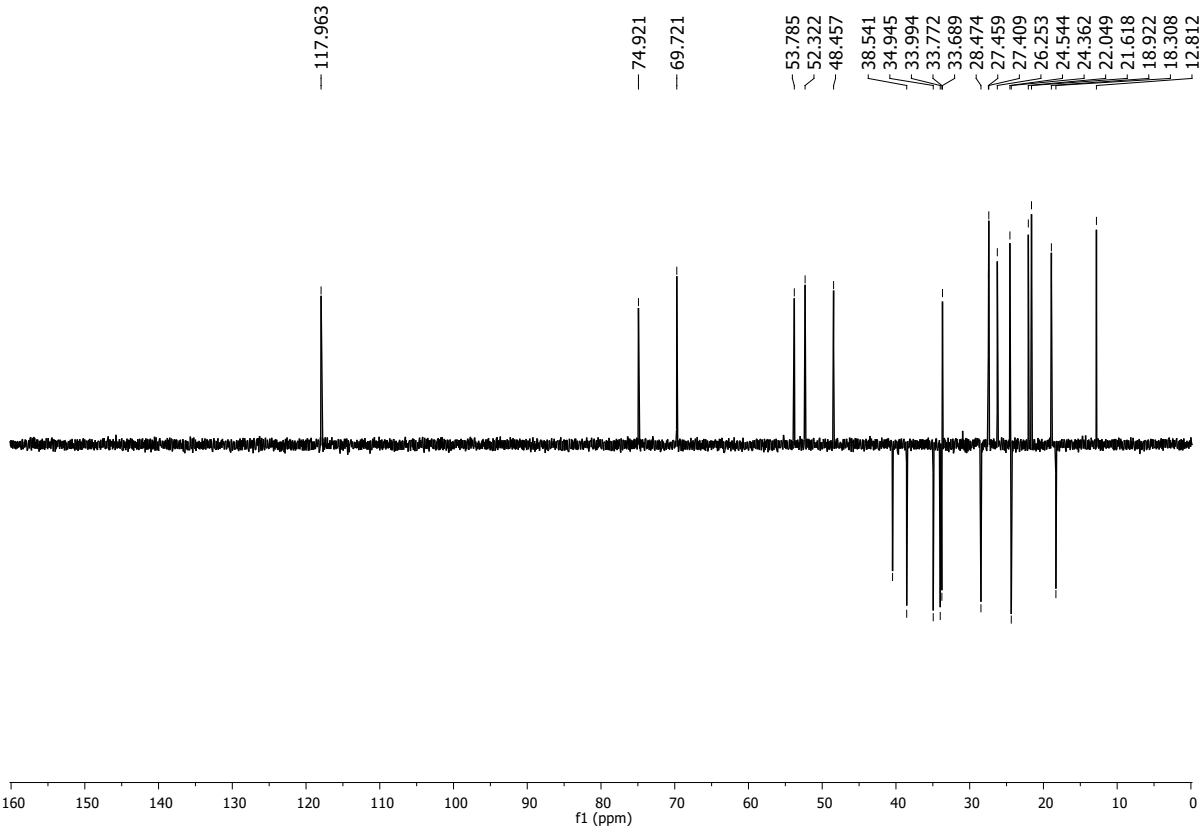


Figure S21. DEPT 135 NMR spectrum of Piscidinol A (3) in $CDCl_3$

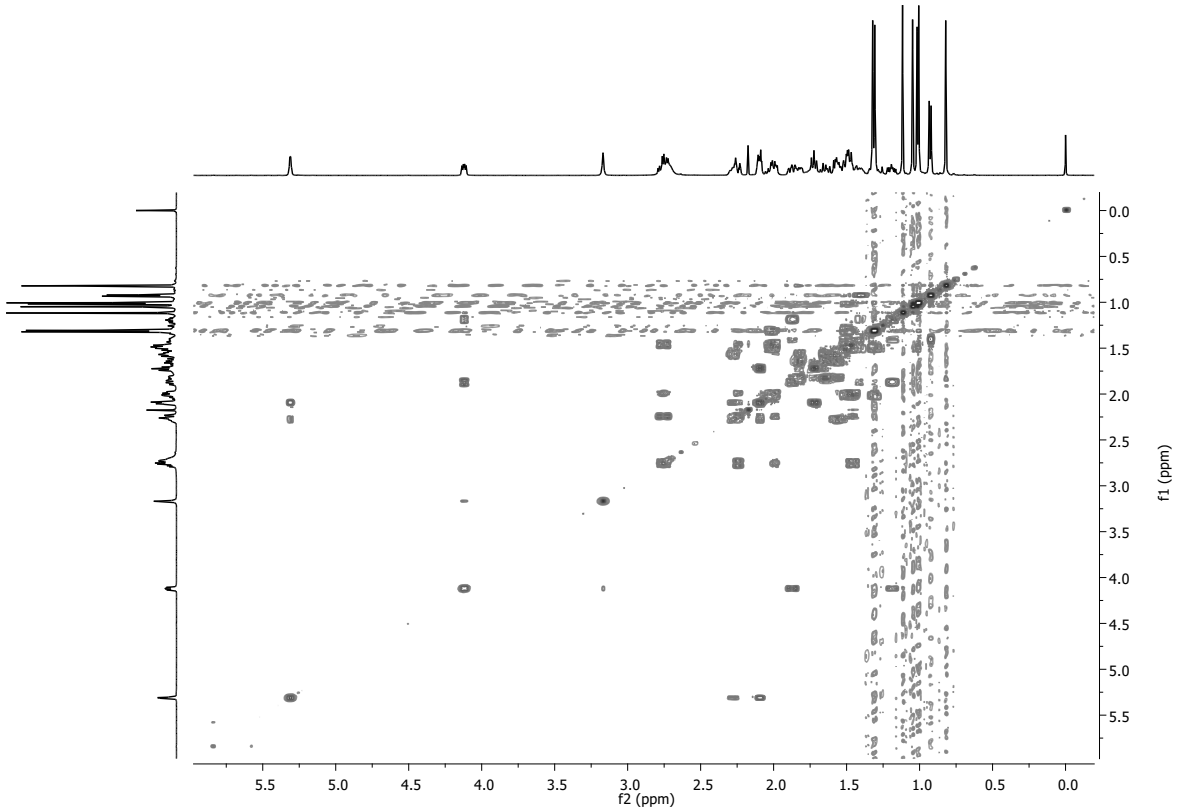


Figure S22. ^{1}H-^{1}H COSY NMR spectrum of Piscidinol A (3) in $CDCl_3$

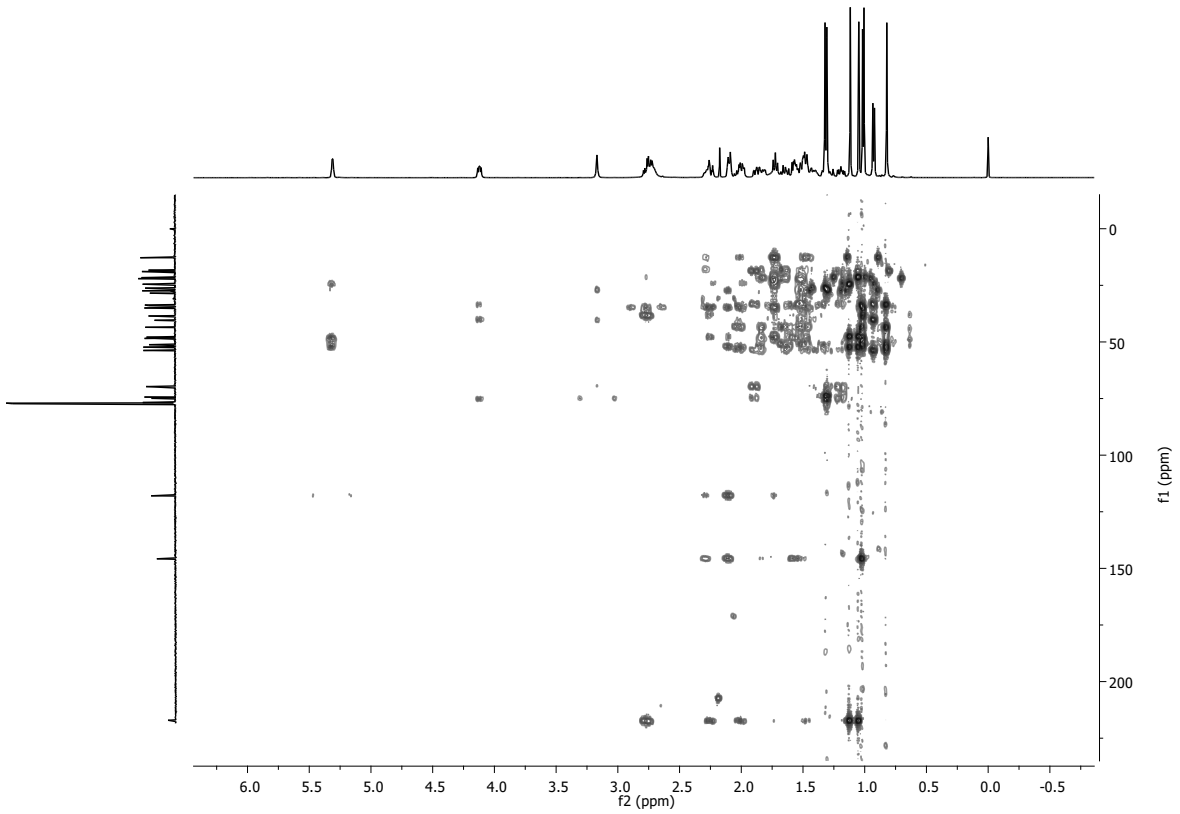


Figure S23. HMBC spectrum of Piscidinol A (3) in $CDCl_3$

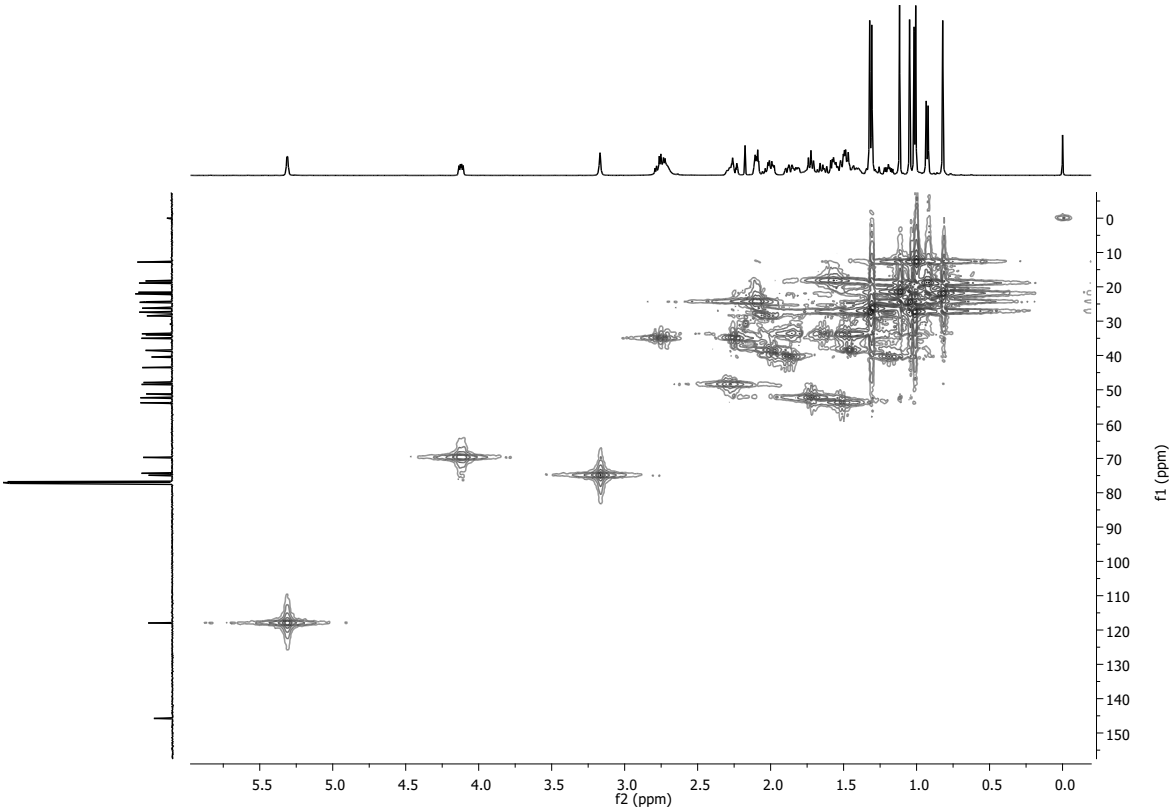


Figure S24. HMQC spectrum of Piscidinol A (3) in $CDCl_3$

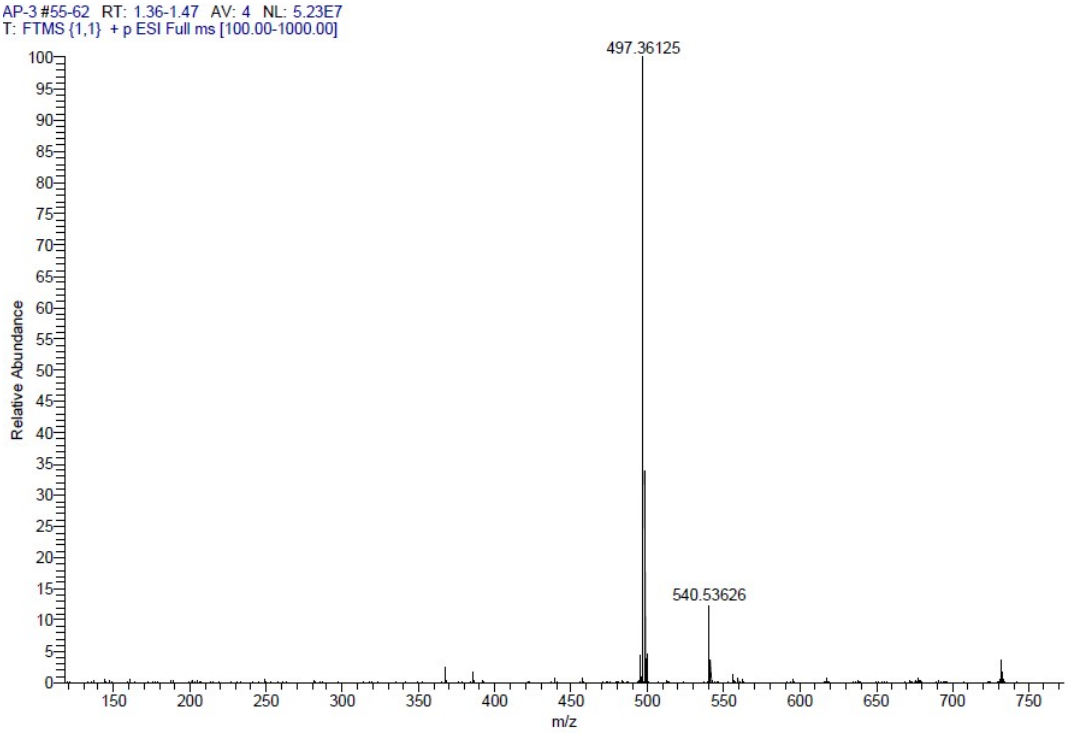


Figure S25. HRMS spectrum of Piscidinol A

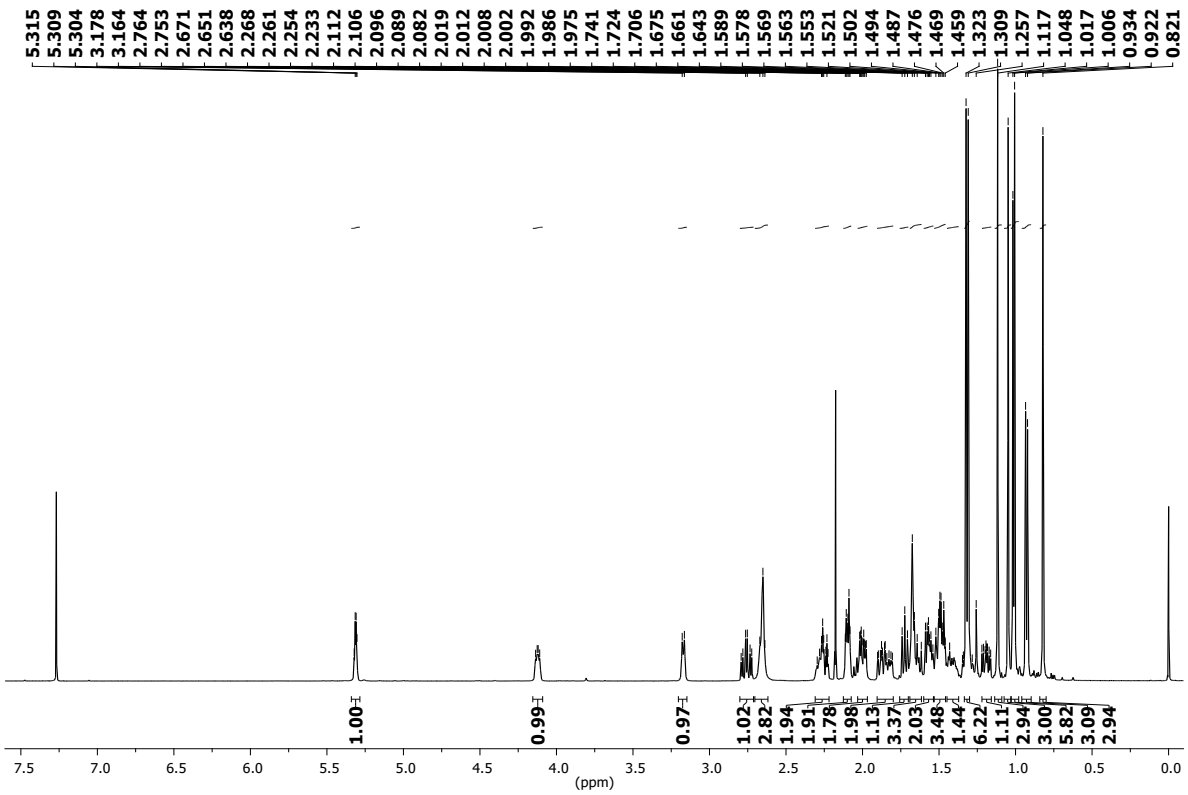


Figure S26. ^{1}H NMR spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

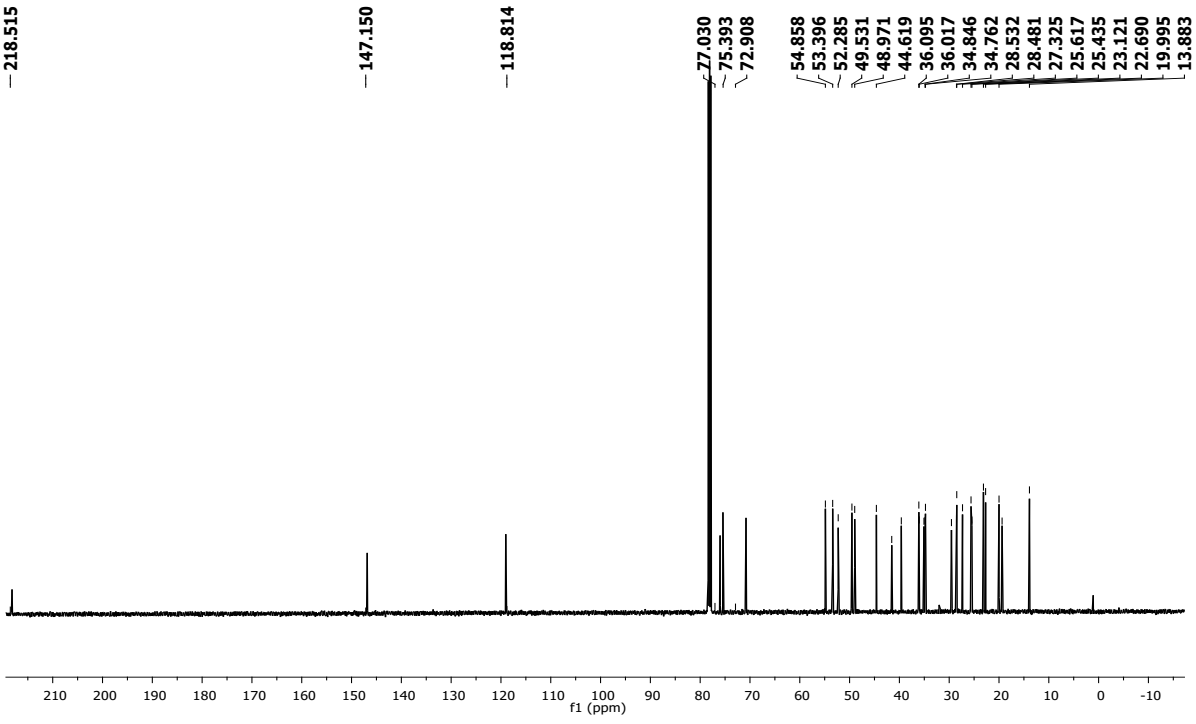


Figure S27. ^{13}C NMR spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

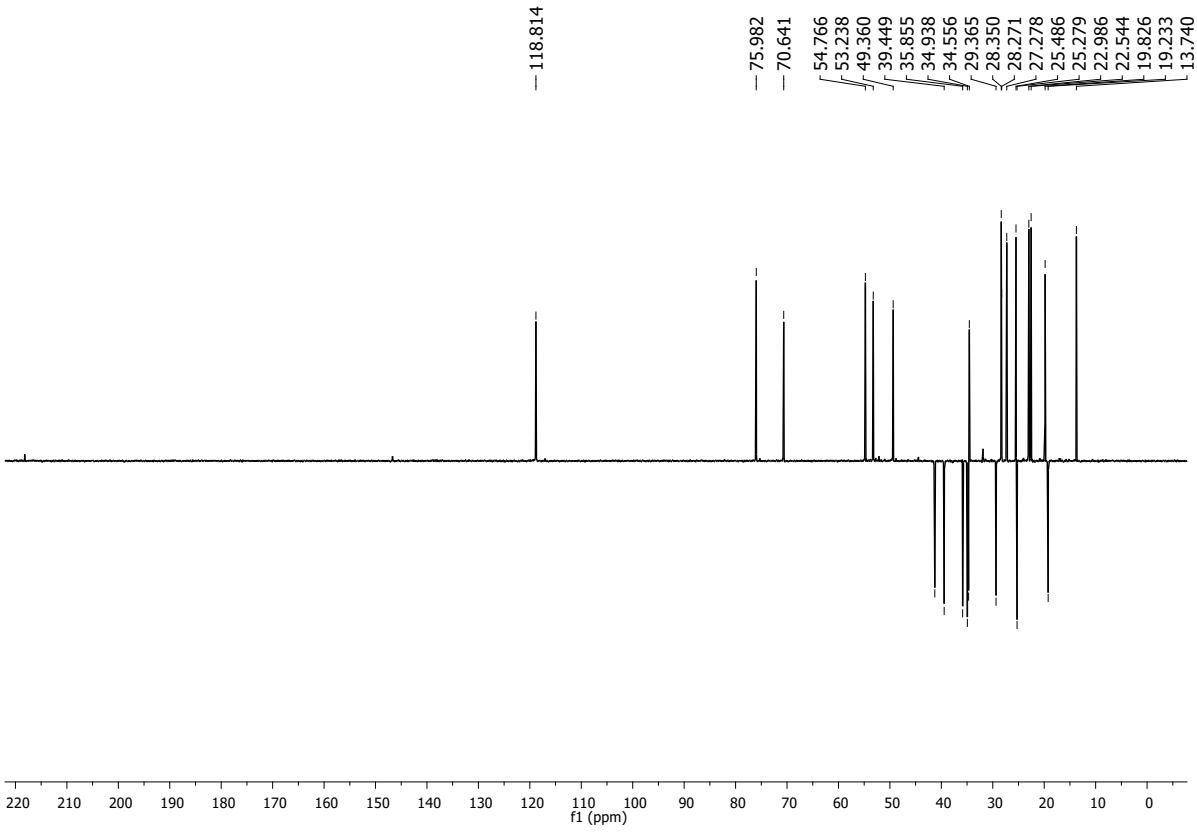


Figure S28. DEPT 135 NMR spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

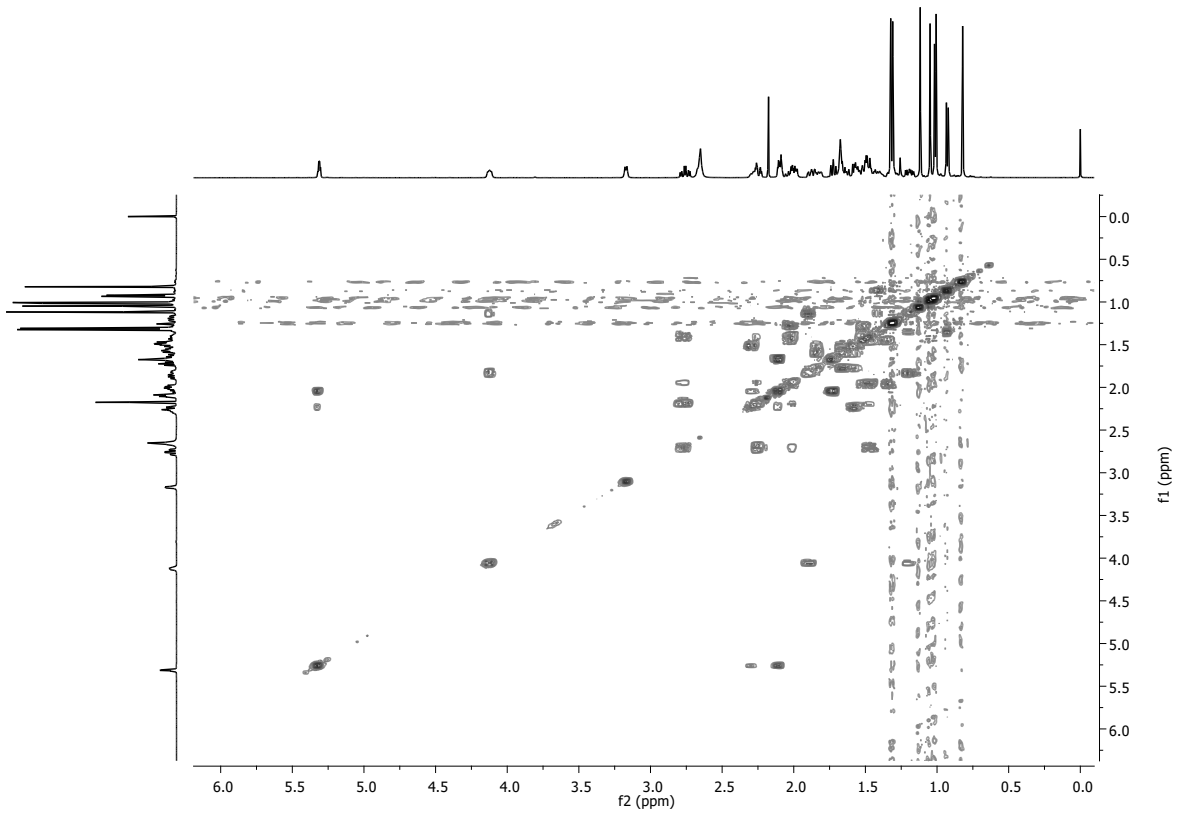


Figure S29. ^{1}H-^{1}H COSY NMR spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

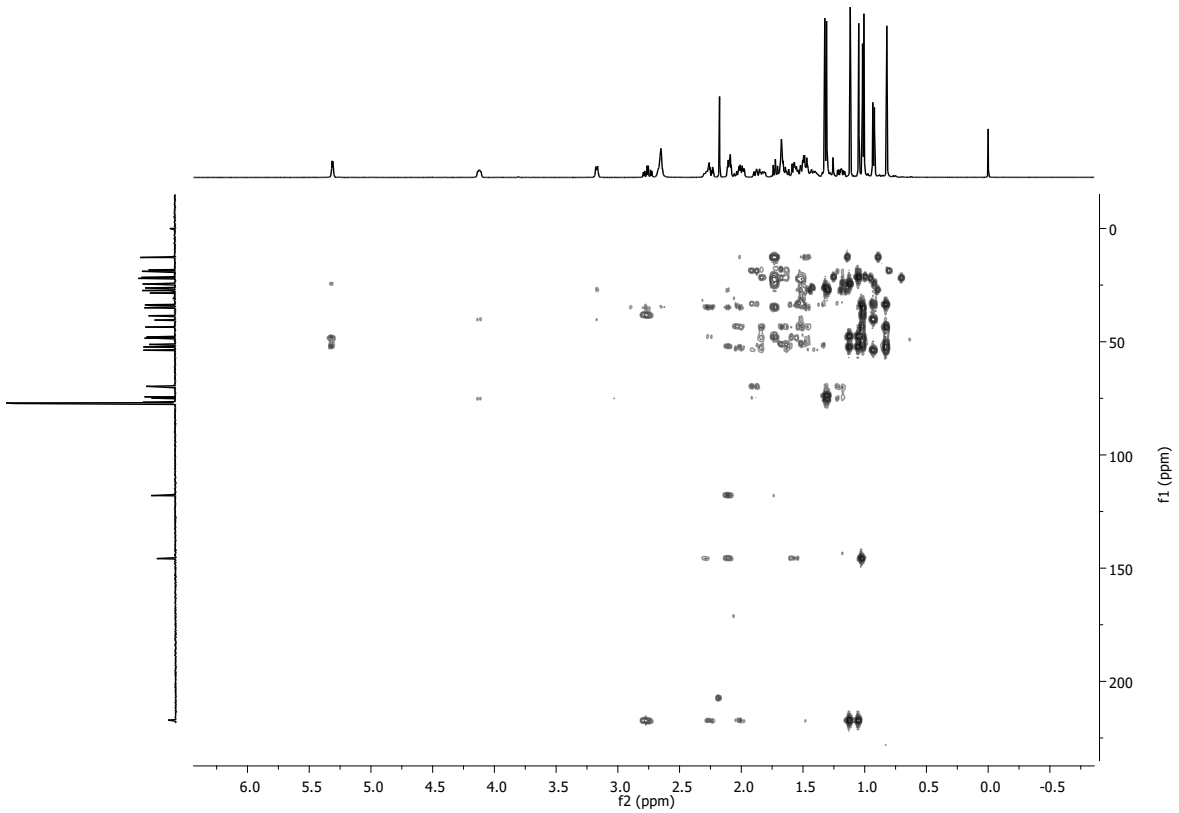


Figure S30. HMBC spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

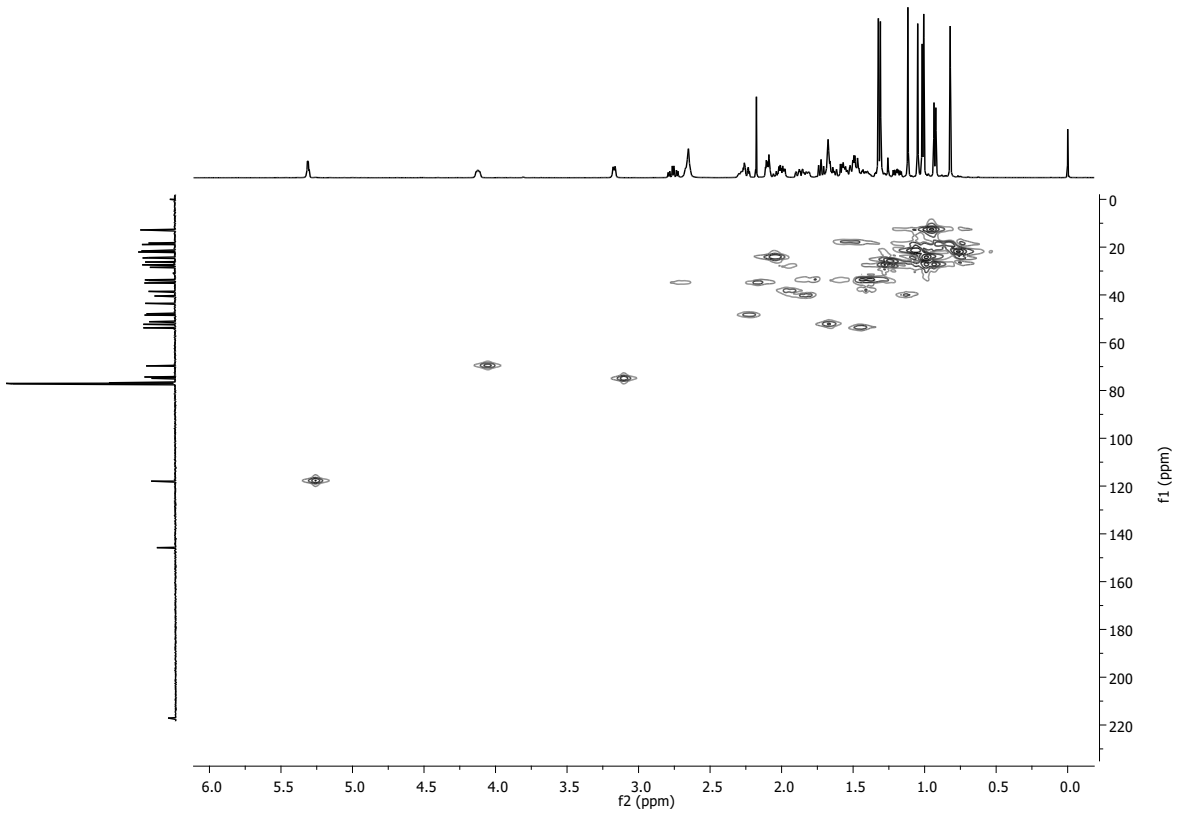


Figure S31. HMQC spectrum of 24-*epi*-Piscidinol A (4) in $CDCl_3$

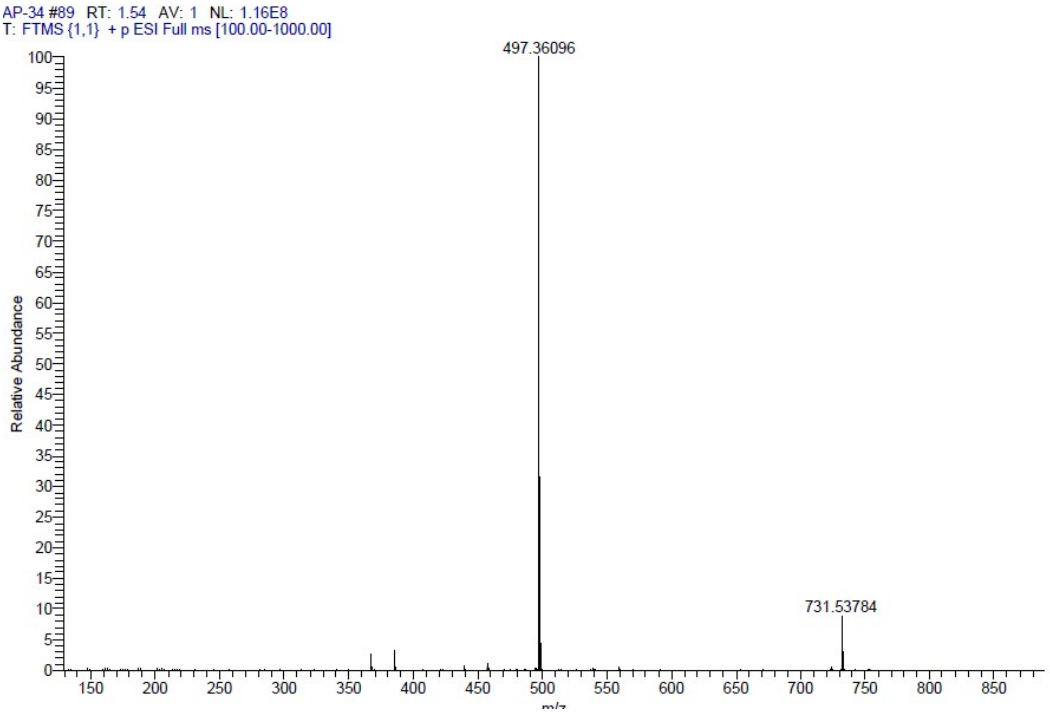


Figure S32. HRMS spectrum of 24-*epi*-Piscidinol A

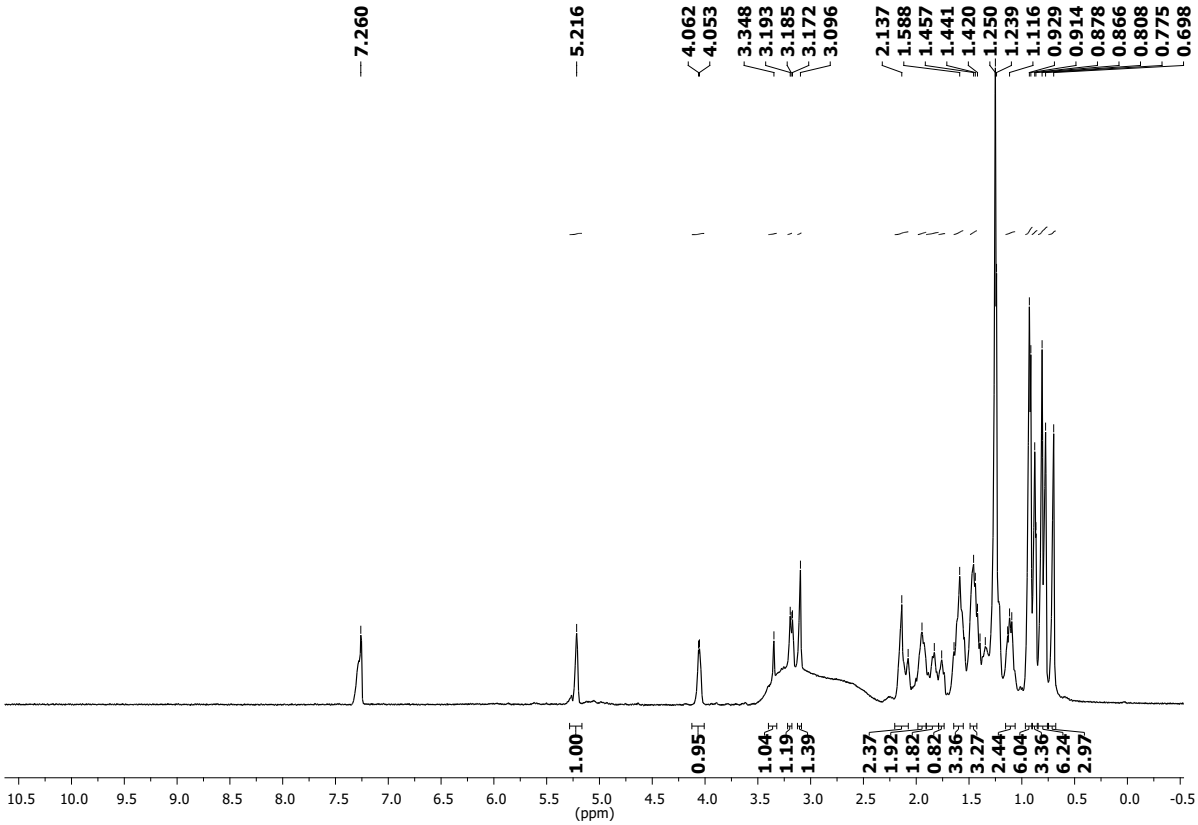


Figure S33. ^{1}H NMR spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

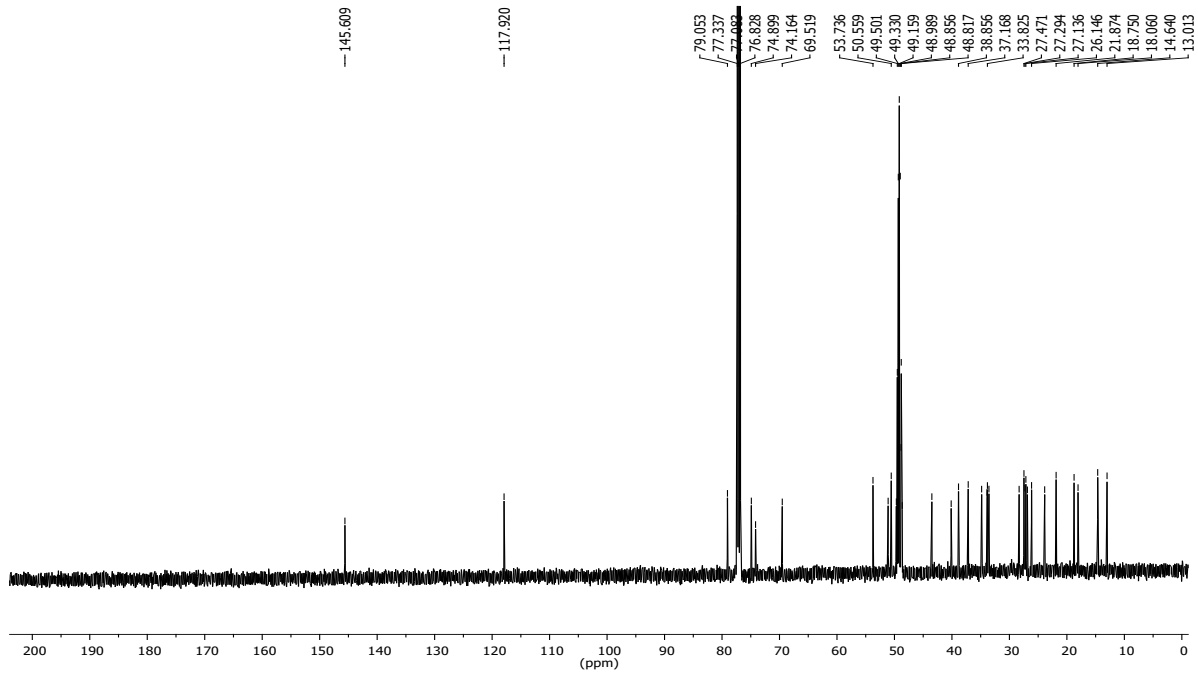


Figure S34. ^{13}C NMR spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

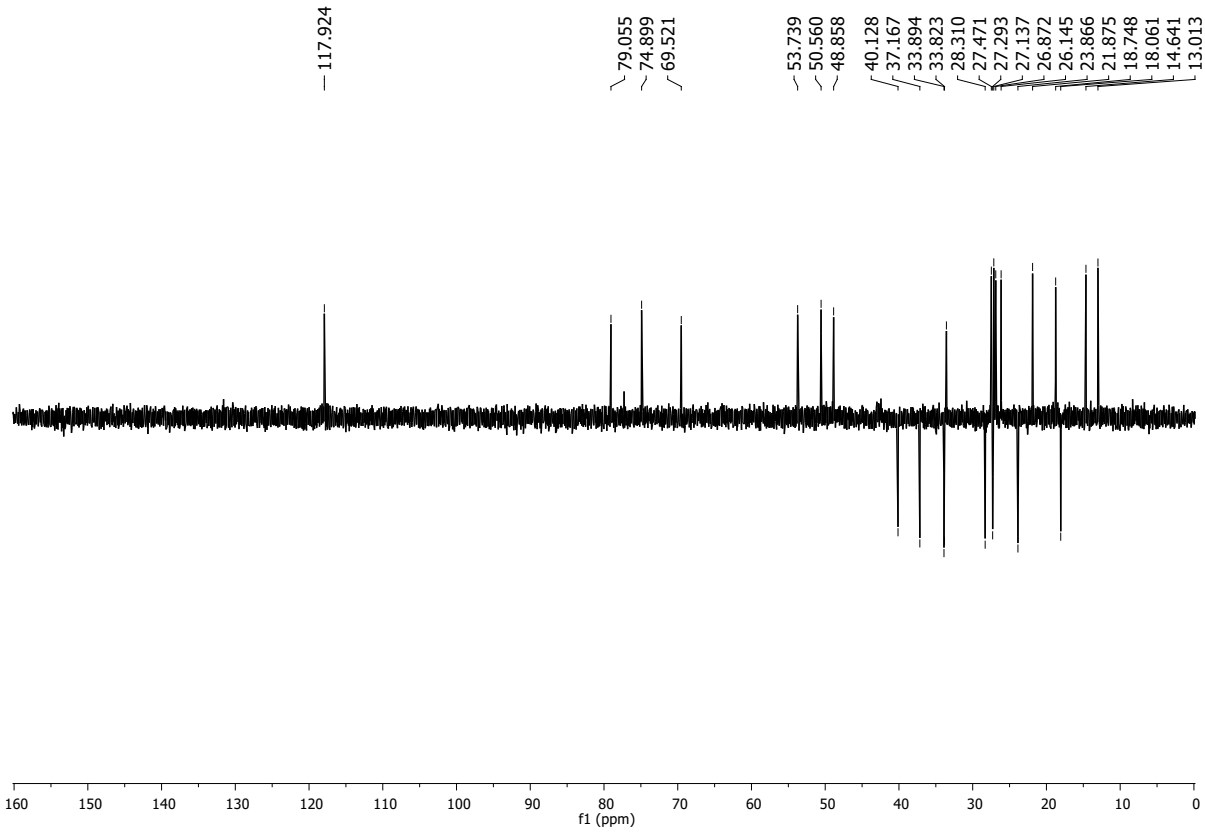


Figure S35. DEPT 135 NMR spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

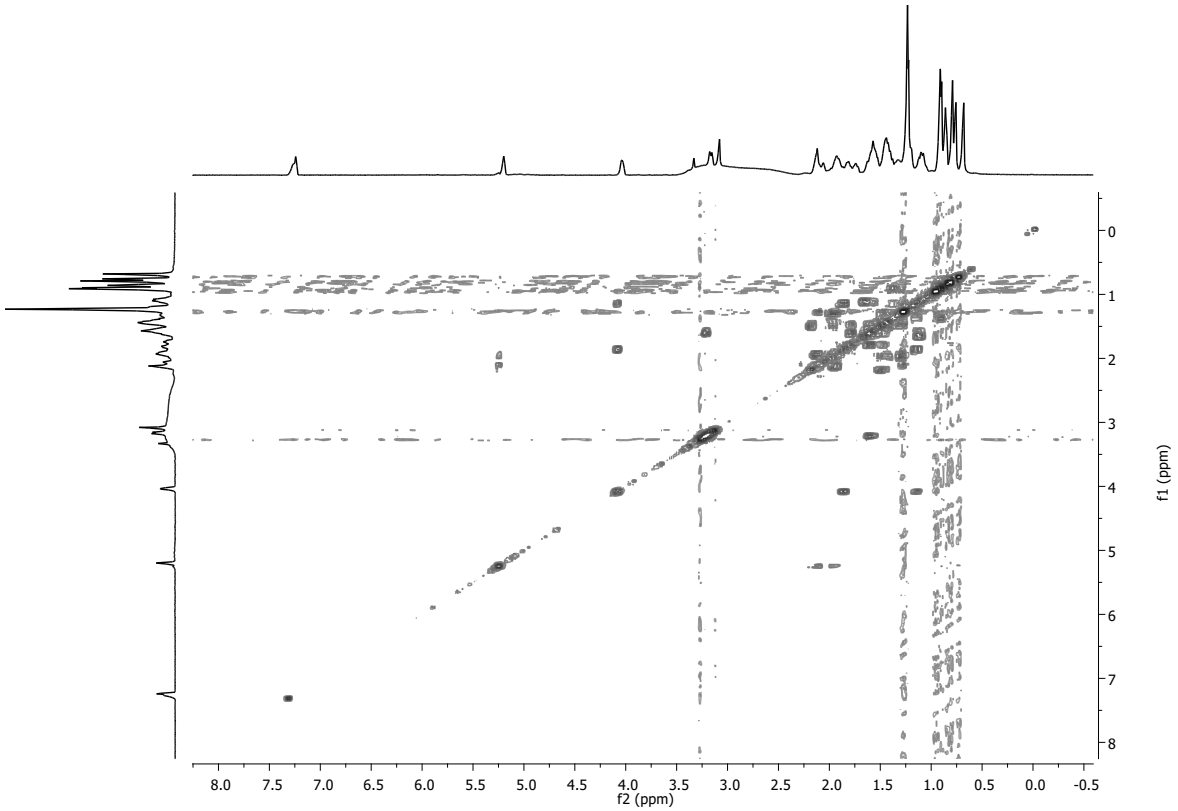


Figure S36. ^{1}H-^{1}H COSY NMR spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

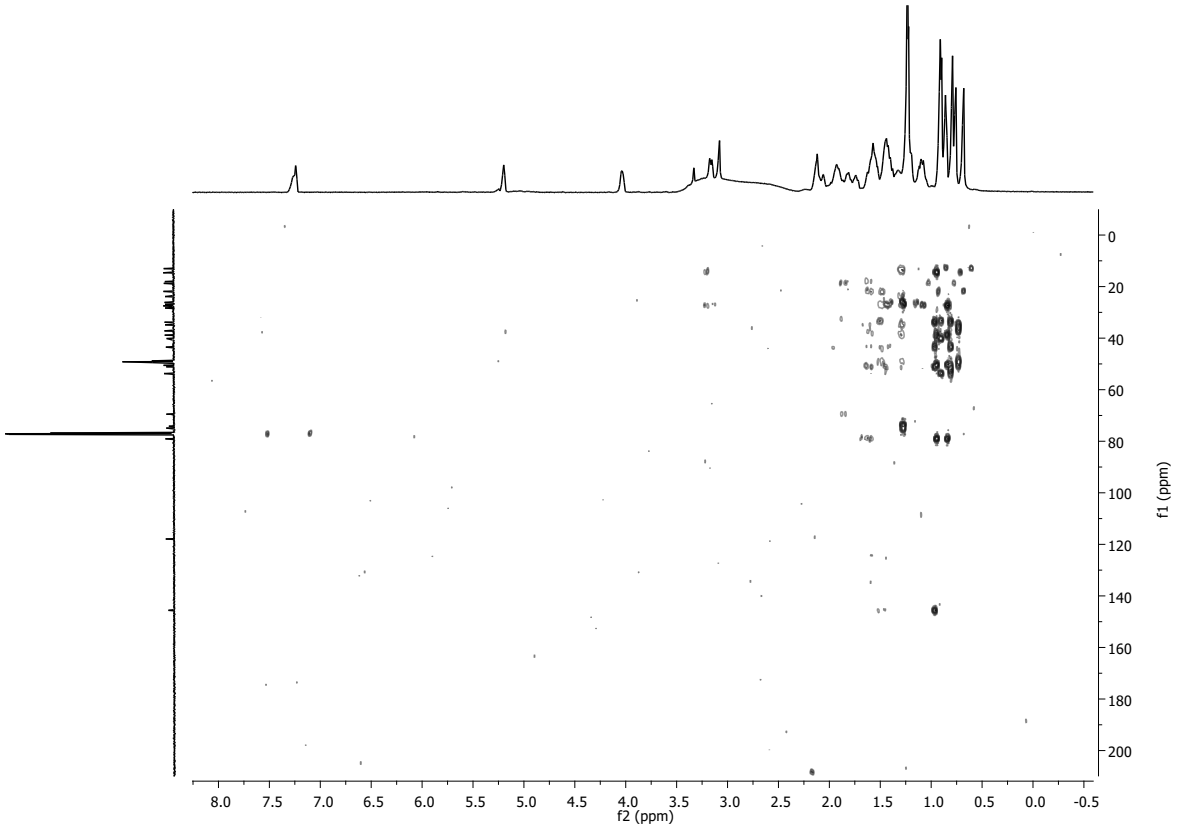


Figure S37. HMBC spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

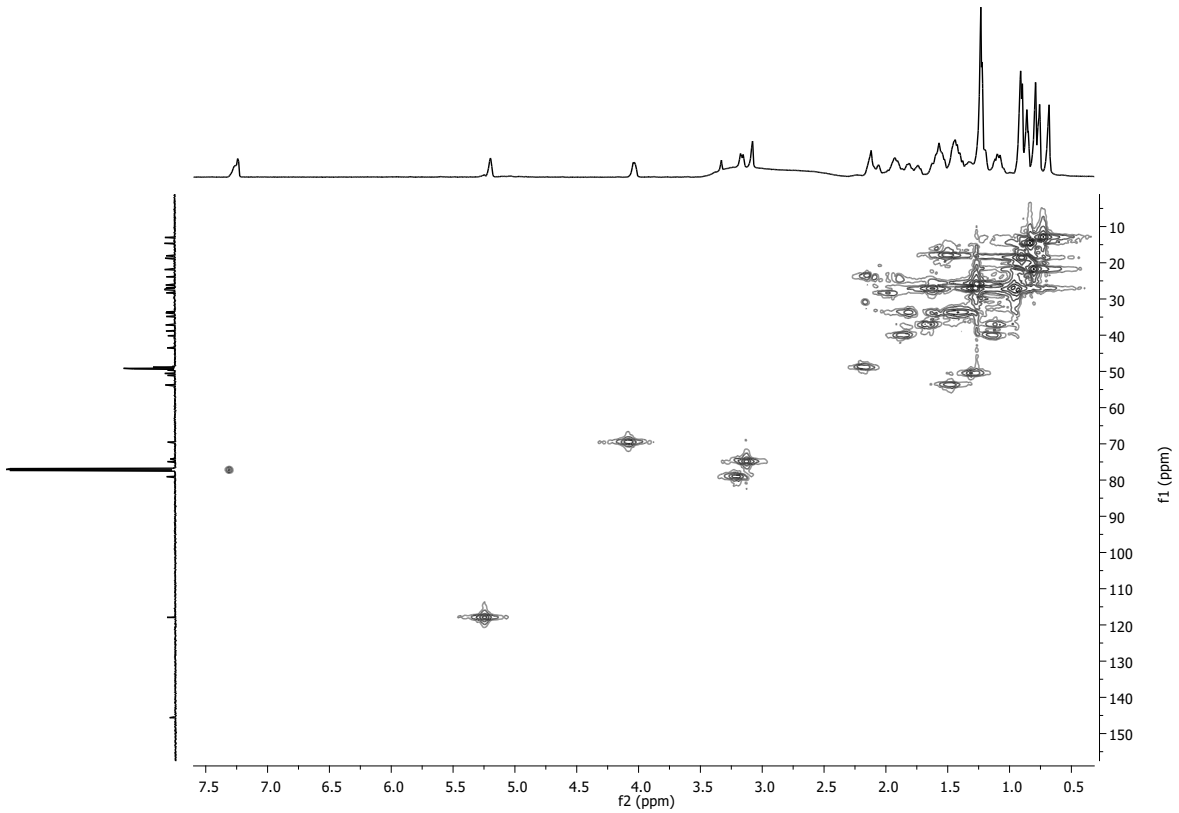


Figure S38. HMQC spectrum of Hispidol A (5) in $CDCl_3$ + MeOD

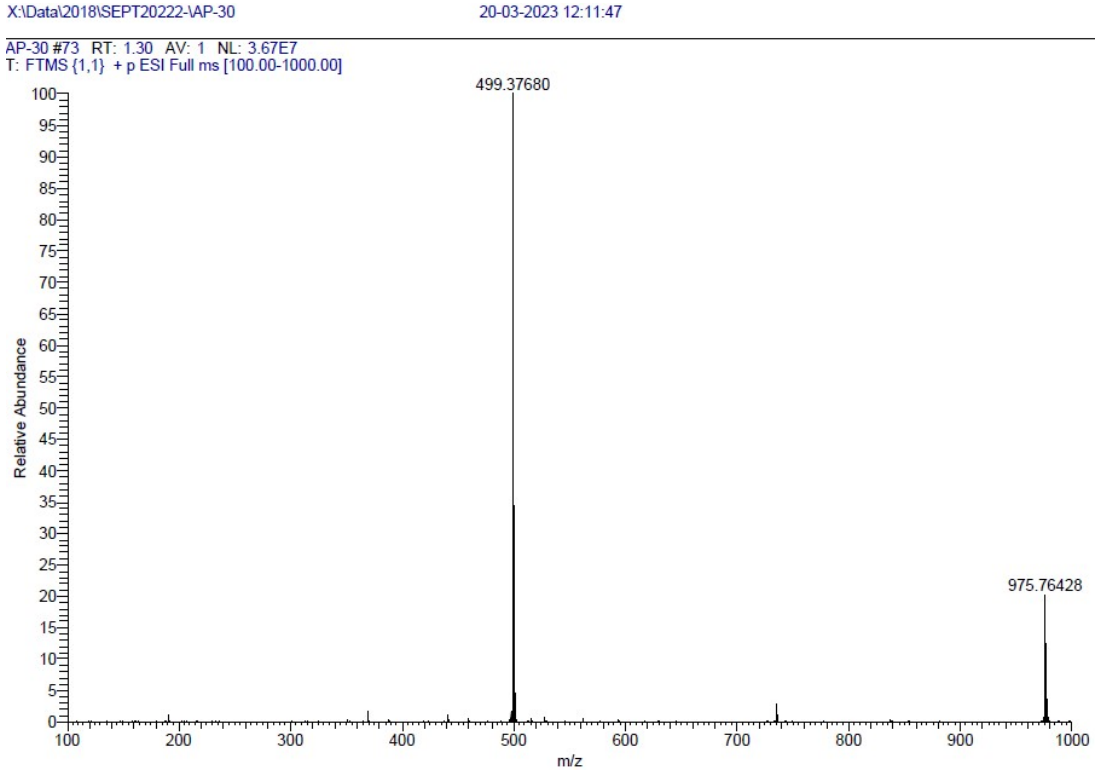
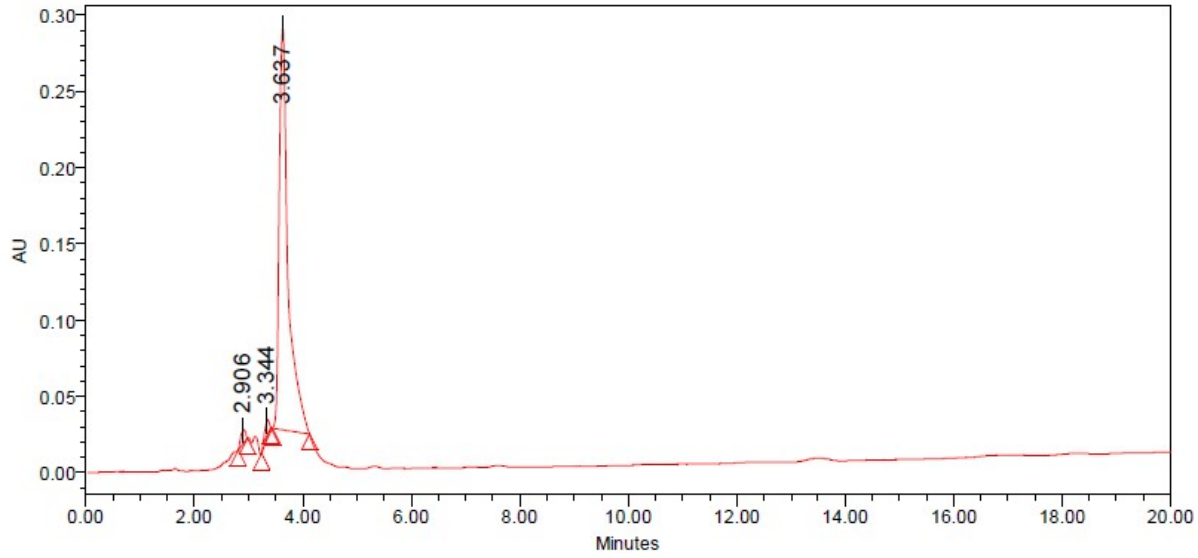


Figure S39. HRMS spectrum of Hispidol A



Processed Channel: 2998 PDA 209.6 nm (2998 (210-400)nm)

	Processed Channel	Retention Time (min)	Area	% Area	Height
1	2998 PDA 209.6 nm (2998 (210-400)nm)	2.906	54608	1.75	8884
2	2998 PDA 209.6 nm (2998 (210-400)nm)	3.344	68752	2.20	11568
3	2998 PDA 209.6 nm (2998 (210-400)nm)	3.637	3000023	96.05	263561

Figure S40. HPLC chromatogram of Hispidol A

Spectral data

Compound 1. Niloticin

Molecular formula: $C_{30}H_{50}O_3$

FT-IR (Neat,υ_{max} cm^{-1}): 3470, 2253, 2145, 1301, 1700 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 5.31 (q, J = 3.5 Hz, 1H, H-7), 3.57 (m, 1H, H-23), 2.76 (td, J = 14.5, 5.5 Hz, 1H, H-2a), 2.66 (d, J = 8.5 Hz, 1H, H-24), 2.31 – 2.27 (m, 1H, H-9), 2.25 (dt, J = 14.2, 3.5 Hz, 1H, H-2b), 2.12 – 2.08 (m, 2H), 2.00 (m, 2H), 1.91 – 1.80 (m, 1H, H-22a), 1.72 (t, J = 8.7 Hz, 1H), 1.64 (m, 1H), 1.59 – 1.54 (m, 2H), 1.53 – 1.45 (m, 5H), 1.44 – 1.38 (m, 1H, H-20), 1.34 (s, 3H, H-27), 1.32 (s, 3H, H-26), 1.24 – 1.16 (m, 1H, H-22b), 1.12 (s, 3H, H-28), 1.05 (s, 3H, H-30), 1.02 (s, 3H, H-29), 1.01 (s, 3H, H-19), 0.95 (d, J = 6.0 Hz, 3H, H-21), 0.81 (s, 3H, H-18) ppm.

^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ 217.0 (C-3), 145.7 (C-8), 118.0 (C-7), 69.2 (C-23), 68.4 (C-24), 60.3 (C-25), 53.2 (C-17), 52.3 (C-5), 51.2 (C-14), 48.4 (C-9), 47.9 (C-4), 43.5 (C-13), 40.6 (C-22), 38.5 (C-1), 35.0 (C-20), 34.9 (C-2), 34.0 (C-15), 33.6 (C-12), 33.5 (C-10), 28.7 (C-16), 27.4 (C-30), 24.8 (C-27), 24.5 (C-6), 24.3 (C-28), 21.7 (C-26), 21.6 (C-18),

19.9 (C-29), 19.8 (C-21), 18.2 (C-11), 12.8 (C-19) ppm.

HRMS (ESI): *m/z* Found 479.3508 $[M+Na]^+$.

Calcld. for $C_{30}H_{48}NaO_3$ is 479.3501.

Compound 2. Bourjotinolone B

Molecular formula: $C_{30}H_{48}O_3$

FT-IR (Neat,υ_{max} cm^{-1}): 3459, 2954, 2930, 1745, 1643, 1440, 1388 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 5.30 (m, 1H, H-7), 5.06 (s, 1H, H-27a), 5.00 (s, 1H, H-27b), 3.89 (t, *J* = 3.5 Hz, 1H, H-24), 3.73 (q, *J* = 5.0 Hz, 1H, H-23), 2.76 (td, *J* = 14.5, 5.5 Hz, 1H, H-2a), 2.66 (d, *J* = 8.5 Hz, 1H, H-24), 2.31 – 2.27 (m, 1H, H-9), 2.25 (dt, *J* = 14.2, 3.5 Hz, 1H, H-2b), 2.12 – 2.08 (m, 2H), 2.00 (m, 2H), 1.91 – 1.80 (m, 1H, H-22a), 1.72 (t, *J* = 8.7 Hz, 1H), 1.64 (m, 1H), 1.59 – 1.54 (m, 2H), 1.53 – 1.45 (m, 5H), 1.44 – 1.38 (m, 1H, H-20), 1.76 (s, 3H, H-26), 1.24 – 1.16 (m, 1H, H-22b), 1.11 (s, 3H, H-28), 1.04 (s, 3H, H-30), 1.01 (s, 3H, H-29), 1.00 (s, 3H, H-19), 0.98 (d, *J* = 6.0 Hz, 3H, H-21), 0.82 (s, 3H, H-18) ppm.

^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ 217.0 (C-3), 145.7 (C-8), 145.0 (C-25), 117.9 (C-7), 113.0 (C-27), 77.2 (C-24), 70.7 (C-23), 53.8 (C-17), 52.4 (C-5), 51.1 (C-14), 48.5 (C-9), 47.9 (C-4), 43.7 (C-13), 39.5 (C-22), 38.5 (C-1), 35.0 (C-20), 34.9 (C-2), 34.5 (C-15), 34.0 (C-12), 33.7 (C-10), 28.4 (C-16), 27.4 (C-30), 24.5 (C-6), 24.3 (C-28), 21.9 (C-26), 21.6 (C-18), 19.5 (C-29), 18.8 (C-21), 18.3 (C-11), 12.8 (C-19) ppm.

HRMS (ESI): *m/z* Found 479.3509 $[M+Na]^+$.

calcld for $C_{30}H_{48}NaO_3$ is 479.3501.

Compound 3. Piscidinol A

Molecular formula: $C_{30}H_{50}O_4$

FT-IR (Neat,υ_{max} cm^{-1}): 3444, 2932, 1730, 1694, 1632, 1454, 1383, 1246 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 5.31 (d, *J* = 3.0 Hz, 1H, H-7), 4.12 (dd, *J* = 9.0, 5.0 Hz, 1H, H-23), 3.17 (s, 1H, H-24), 2.76 (td, *J* = 14.5, 5.5 Hz, 1H, H-2a), 2.31 – 2.27 (m, 1H, H-9), 2.25 (dt, *J* = 14.2, 3.5 Hz, 1H, H-2b), 2.12 – 2.08 (m, 2H), 2.00 (m, 2H), 1.91 – 1.80 (m, 1H, H-22a), 1.72 (t, *J* = 8.7 Hz, 1H), 1.64 (m, 1H), 1.59 – 1.54 (m, 2H), 1.53 – 1.45 (m, 5H), 1.44 – 1.38 (m, 1H, H-20), 1.32 (s, 3H, H-26), 1.31 (s, 3H, H-27), 1.24 – 1.16 (m, 1H, H-22b), 1.12 (s, 3H, H-28), 1.05 (s, 3H, H-30), 1.02 (s, 3H, H-29), 1.01 (s, 3H, H-19), 0.93 (d, *J* = 6.4 Hz, 3H, H-21), 0.82 (s, 3H, H-18) ppm.

^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ 217.0 (C-3), 145.7 (C-8), 117.9 (C-7), 74.9 (C-24), 74.3 (C-25), 69.7 (C-23), 53.7 (C-17), 52.3 (C-5), 51.2 (C-14), 48.4 (C-9), 47.8 (C-4), 43.5 (C-13), 40.4 (C-22), 38.5 (C-1), 35.0 (C-20), 34.9 (C-2), 33.9 (C-15), 33.7 (C-12), 33.6 (C-10), 28.4 (C-16), 27.4 (C-30), 27.4 (C-27), 26.2 (C-6), 24.5 (C-28), 24.3 (C-26), 22.0 (C-18), 21.6 (C-29), 18.9 (C-21), 18.3 (C-11), 12.8 (C-19) ppm.

HRMS (ESI): *m/z* Found 497.3612

Calcld. for $C_{30}H_{50}NaO_4$ is 497.3607.

Compound 4. 24-*epi*-piscidinol A

Molecular formula: $C_{30}H_{50}O_4$

FT-IR (Neat,υ_{max} cm^{-1}): 3451, 2948, 1731, 1637, 1450, 1444, 1376 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 5.31 (d, J = 3.0 Hz, 1H, H-7), 4.12 (dd, J = 9.0, 5.0 Hz, 1H, H-23), 3.17 (d, J = 7.0 Hz, 1H, H-24), 2.76 (td, J = 14.5, 5.5 Hz, 1H, H-2a), 2.72 – 2.68 (br s, 3OH, OH-23, OH-24 & OH-25), 2.31 – 2.27 (m, 1H, H-9), 2.25 (dt, J = 14.2, 3.5 Hz, 1H, H-2b), 2.12 – 2.08 (m, 2H), 2.00 (m, 2H), 1.91 – 1.80 (m, 1H, H-22a), 1.72 (t, J = 8.7 Hz, 1H), 1.64 (m, 1H), 1.59 – 1.54 (m, 2H), 1.53 – 1.45 (m, 5H), 1.44 – 1.38 (m, 1H, H-20), 1.32 (s, 3H, H-26), 1.31 (s, 3H, H-27), 1.24 – 1.16 (m, 1H, H-22b), 1.12 (s, 3H, H-28), 1.05 (s, 3H, H-30), 1.02 (s, 3H, H-29), 1.01 (s, 3H, H-19), 0.93 (d, J = 6.4 Hz, 3H, H-21), 0.82 (s, 3H, H-18) ppm.

^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ 218.5 (C-3), 147.1 (C-8), 118.8 (C-7), 77.0 (C-24), 75.3 (C-25), 72.9 (C-23), 54.8 (C-17), 53.3 (C-5), 52.2 (C-14), 49.5 (C-9), 48.9 (C-4), 44.6 (C-13), 41.5 (C-22), 39.6 (C-1), 36.1 (C-20), 36.0 (C-2), 35.0 (C-15), 34.8 (C-12), 34.7 (C-10), 29.5 (C-16), 28.5 (C-30), 28.4 (C-27), 27.3 (C-6), 25.6 (C-28), 25.4 (C-26), 23.1 (C-18), 22.6 (C-29), 19.9 (C-21), 19.3 (C-11), 13.8 (C-19) ppm.

HRMS (ESI): *m/z*. Found 497.3609.

Calcld. for $C_{30}H_{50}NaO_4$ is 497.3607

Compound 5. Hispidol A

Molecular formula: $C_{30}H_{52}O_4$

FT-IR (Neat,υ_{max} cm^{-1}): 3453, 2970, 1731, 1642, 1463, 1385, 1247 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$+2 drops MeOD, TMS): δ 5.26 (m, H-7), 4.05 (m, 1H, H-23), 3.18 (m, 1H, H-3), 3.09 (s, 1H, H-24), 2.17 (m, 1H, H-2), 2.15 (m, 1H, H-9), 2.00 (m, 1H),

1.87 (m, 1H), 2.00 (m, 2H), 1.81 (m, 1H), 1.65 (m, 1H), 1.62 (m, 2H), 1.50 (m, 1H), 1.41 (m, 5H), 1.40 (m, 1H), 1.25 (s, 3H, H-27), 1.23 (s, 3H, H-26), 1.16 (m, 1H), 0.92 (s, 3H, H-28), 0.91 (d, *J*= 6.0 Hz, 3H, H-21), 0.86 (s, 3H, H-29), 0.80 (s, 3H, H-30), 0.77 (s, 3H, H-19), 0.69 (s, 3H, H-18) ppm

^{13}C NMR (126 MHz, $CDCl_3$, TMS): δ 145.6 (C-8), 117.9 (C-7), 79.0 (C-3), 74.8 (C-24), 74.1 (C-25), 69.5 (C-23), 53.7 (C-17), 51.1 (C-14), 51.7 (C-5), 51.1 (C-9), 50.5 (C-13), 43.4 (C-22), 40.1 (C-4), 38.8 (C-1), 37.1 (C-10), 34.8 (C-20), 33.9 (C-12), 33.8 (C-15), 33.5 (C-16), 28.3 (C-2), 27.4 (C-28), 27.2 (C-6), 27.1 (C-30), 26.8 (C-27), 26.1 (C-26), 23.8 (C-6), 21.8 (C-19), 18.7 (C-21), 18.4 (C-11), 14.6 (C-29), 13.0 (C-18) ppm.

HRMS (ESI): *m/z*. Found 499.3768.

Calcld. for $C_{30}H_{52}NaO_4$ is 499.3763

4. Preliminary cytotoxicity evaluation of niloticin in SiHa cell line

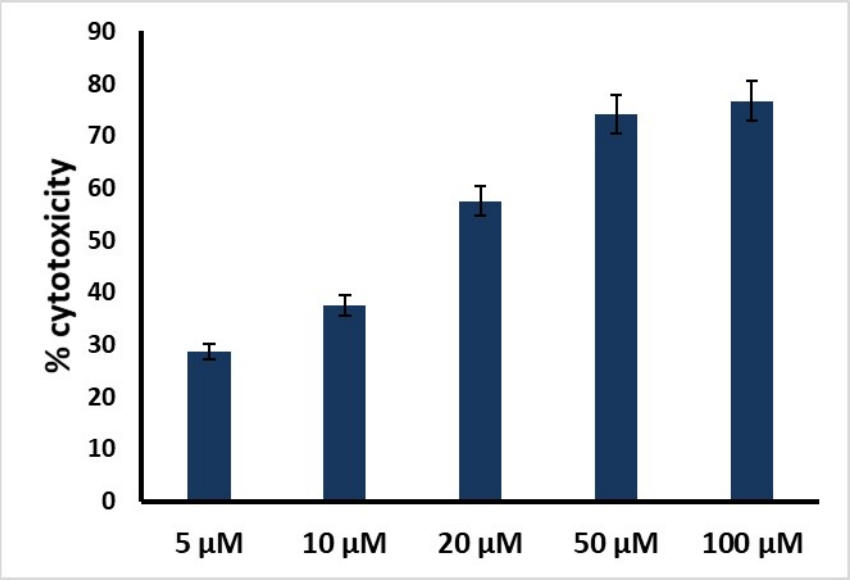


Figure S41: Percentage inhibition of niloticin at 24 hours in SiHa cells

5. Molecular docking of niloticin with various apoptotic proteins

Entry	PDB ID	Docking Score	Interacting Residues
1	1TUP	-7.2	ASN 263, GLY 262, GLU 258, LUE 264, THR 256, TYR103, ARG 267, ILE 254, THR 256, SER 99, PRO 98, SER 95, MET 160, ARG 158
2	3EZQ	-7.1	GLN 244, GLY247, PHE 248, LYS 251, ASN 252, GLU 289, ASP 292, THR 293, LYS 296, ASP 297, LYS 300, ALA 301
3	5L19	-6.8	ARG 144, VAL 146, ALA 147, HIS 148, LEU 168, LEU 170, TYR 192, PHE 275, Leu 278, TYR 279, LEU 278
4	6EB6	-7.7	ARG 89, PHE 92, PHE 93, ALA 96, ALA 97, PHE 100, SER 101, ALA 139, ASP 142, PHE 143, GLU 146, ARG 147
5	2UZE	-7.2	PHE 152, GLY 153, VAL 154, PRO 155, VAL 156, GLU 172, CYS 177, TYR 179, SER 181, THR 182, Met 233, GLY 229, TRP 227, PRO 228, ASP 270, PRO 271, ASN 272
6	6O0K	-9.0	PHE 104, SER 105, TYR 108, ASP 111, MET 115, VAL 133, LEU 137, ASN 143, GLY 145 ARG 146, ALA 149, PHE 150, GLU 152, PHE 153, VAL 156
7	1TNR	-7.2	HIS 32, PHE 74, TYR 76, TYR 134, PRO 161, SER 162, PHE 165, PHE 169, LEU 171

Table S2. Docking scores and interacting residues of selected proteins in apoptotic pathway with niloticin

6.

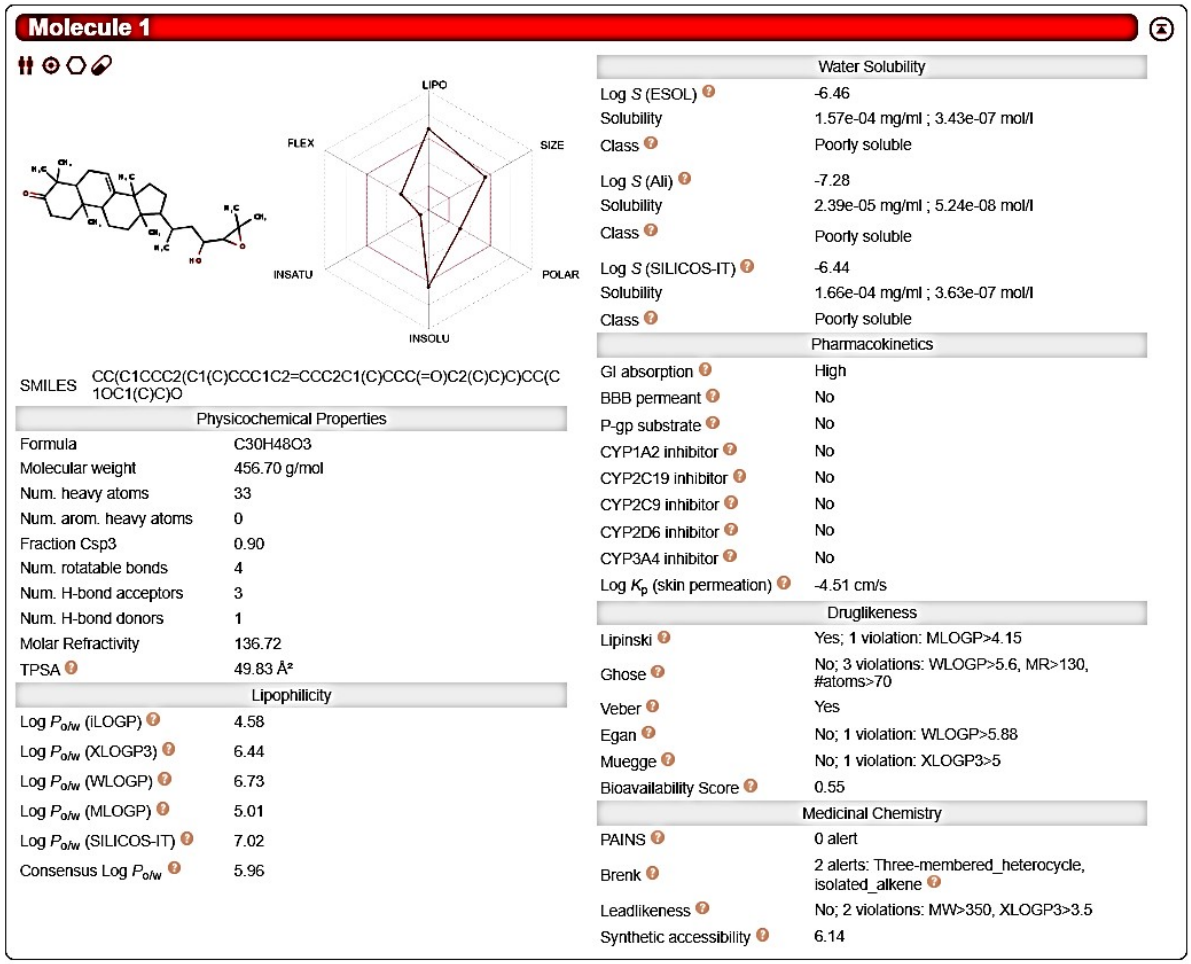


Figure S42. Properties of niloticin

7. Cell cycle regulatory protein expression

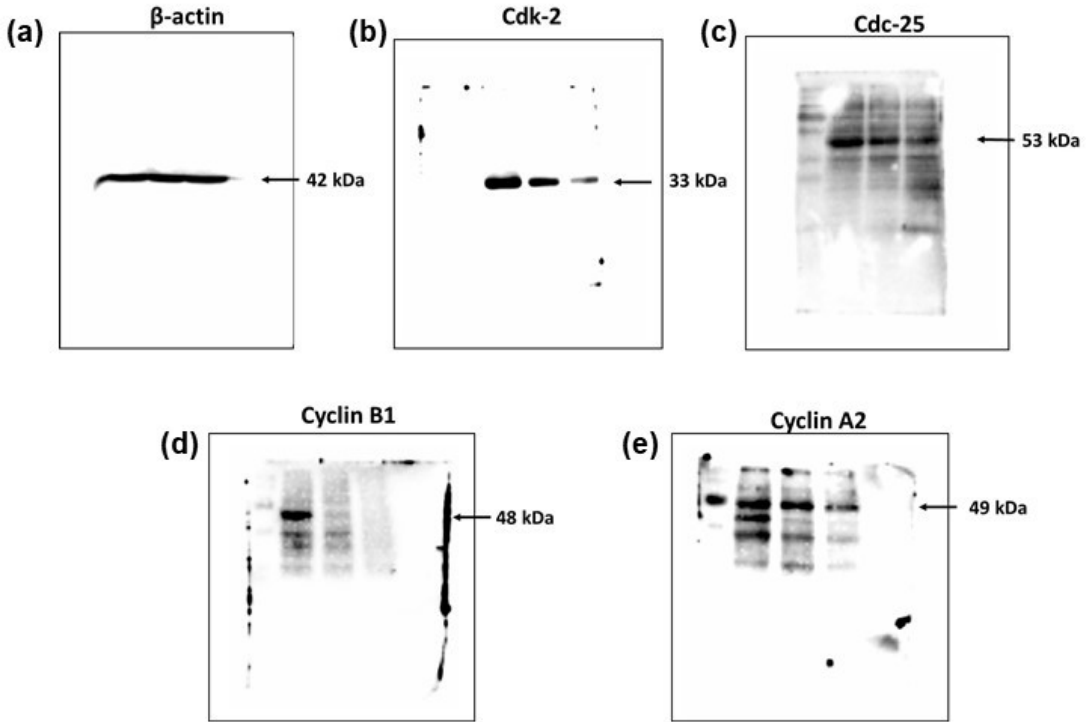


Figure S43. Full blot images of a) β-actin, b) Cdk-2, c) Cdc-25, d) Cyclin B1, e) Cyclin A2 in the order of control (lane 1), 7 µM (lane 2), 11 µM (lane 3).