Supporting Information

Waikialoid A Suppresses Hyphal Morphogenesis and Inhibits Biofilm Development in Pathogenic *Candida albicans*

Xiaoru Wang,[†,‡] Jianlan You,[†,‡] Jarrod B. King,[†] Douglas R. Powell,[§] Robert H. Cichewicz*[,†,⊥]

Natural Products Discovery Group, Department of Chemistry and Biochemistry, Stephenson Life Sciences Research Center 101 Stephenson Parkway, Room 1000, University of Oklahoma, Norman, Oklahoma, 73019-5251, USA.

Ecology and Evolutionary Biology Program, University of Oklahoma, Norman, Oklahoma, 73019-5251, USA.

[†]Natural Products Discovery Group, Department of Chemistry and Biochemistry.

[‡]These authors contributed equally to this work.

[§]Department of Chemistry and Biochemistry.

[⊥]Ecology and Evolutionary Biology Program.

Figure S17. FTIR spectrum of waikialoid B (**14**).

Figure S18. CD spectrum of waikialoid B (**14**).

Figure S19. HRESIMS of waikialide A **(15).**

Figure S20. ^{1}H-NMR spectrum (400 MHz, CD_3OD) waikialide A **(15).**

Figure S21. ^{13}C-NMR spectrum (100 MHz, CD_3OD) waikialide A **(15).**

Figure S22. HSQC-NMR spectrum (400 MHz, CD_3OD) waikialide A **(15).**

Figure S23. HMBC-NMR spectrum (400 MHz, CD_3OD) waikialide A **(15).**

Figure S24. COSY-NMR spectrum (400 MHz, CD_3OD) of waikialide A **(15).**

Figure S25. NOESY-NMR spectrum (400 MHz, CD_3OD) of waikialide A **(15).**

Figure S26. FTIR spectrum of waikialide A **(15).**

Figure S27. ORTEP structure for **15** generated from the X-ray diffraction data.

Figure S28. CD spectrum of waikialide A **(15).**

Figure S29. Octant rule applied to waikialide A **(15).**

Figure S30 ^{1}H -NMR (500 MHz, pyrindine-d_5) of derivative of waikialide A (**15a**).

Figure S31. ^{1}H -NMR (500 MHz, pyrindine-d_5) of derivative of waikialide A (**15b**).

Figure S32. HRESIMS of waikialide B **(16).**

Figure S33. ^{1}H-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

Figure S34. ^{13}C-NMR spectrum (100 MHz, CD_3OD) waikialide B **(16).**

Figure S35. HSQC-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

Figure S36. HMBC-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

Figure S37. COSY-NMR spectrum (400 MHz, CD_3OD) of waikialide B **(16).**

Figure S38. NOESY-NMR spectrum (400 MHz, CD_3OD) of waikialide B **(16).**

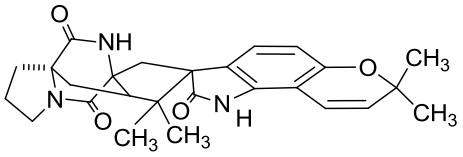
Figure S39. ^{1}H-^{1}H NOESY correlations (dashed double-headed arrows) used to corroborate the relative configuration of the substructure in metabolite **16**.

Figure S40. FTIR spectrum of waikialide B **(16).**

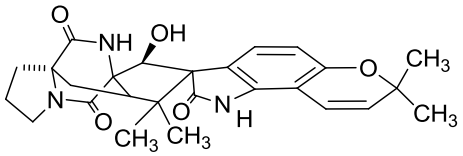
Figure S41. Effects of compounds **1** and **15** on *Candida albicans* hyphae formation.

Figure S42. Time of addition assay of compound **1** (3 µM).

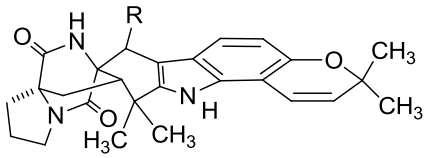
Figure S43. Time of addition assay of compound **1** (25 µM).



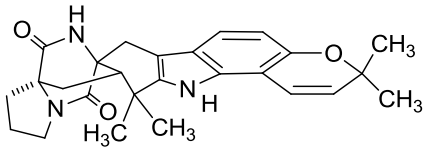
2 notoamide B



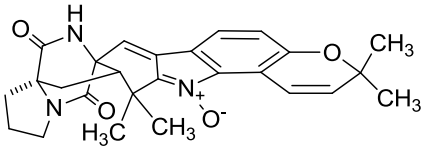
3 sclerotiamide



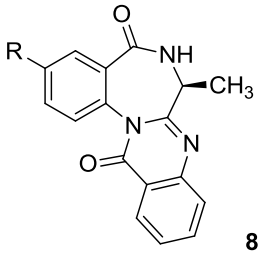
4 notoamide F R = OCH_3
5 notoamide R R = OH



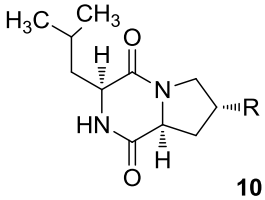
6 stephacidin A



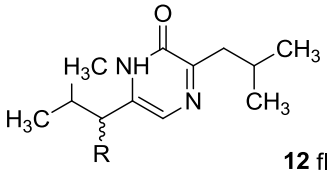
7 avrainvillamide



8 circumdatin C R = OH
9 circumdatin F R = H

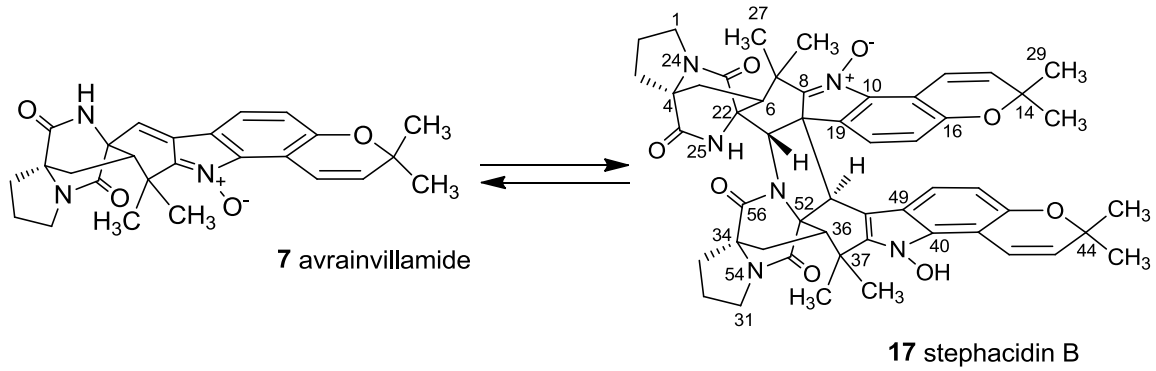


10 R = H
11 R = OH



12 flavacol R = H
13 R = OH

Scheme S1. Structures of compound **2-14**



Scheme S2. Interconversion of avrainvillamide (**7**) and stephacidin B (**17**)

Table S1. Crystal data and structure refinement for waikialoid A (**1**)

Empirical formula	$(C_{52}\ H_{54}\ N_6O_7) \cdot (H_2\ O) \cdot (C\ H_4\ O)$
	$C_{53}\ H_{60}\ N_6\ O_9$
Formula weight	925.07
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 10.6276(4) Å α= 90°
	b = 17.6706(6) Å β= 104.187(2)°
	c = 12.8917(4) Å γ= 90°
Volume	2347.17(14) $Å^3$
Z, Z'	2, 1
Density (calculated)	1.309 Mg/m^3
Wavelength	1.54178 Å
Temperature	100(2) K
F(000)	984
Absorption coefficient	0.731 mm^{-1}
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8418 and 0.6520
Theta range for data collection	3.54 to 67.16°
Reflections collected	67703
Independent reflections	7859 [R(int) = 0.0272]
Data / restraints / parameters	7859 / 21 / 635
wR (F^2 all data)	$wR2$ = 0.0845
R(F obsd data)	$R1$ = 0.0316
Goodness-of-fit on F^2	1.005
Observed data [I > 2σ(I)]	7855
Absolute structure parameter	0.05(11)
Largest and mean shift / s.u.	0.033 and 0.001
Largest diff. peak and hole	0.343 and -0.281 e/$Å^3$

Table S2. Crystal data and structure refinement for waikialide A **(15)**

Empirical formula	C_{14} H_{20} O_2
Formula weight	220.30
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 7.274(8) Å α= 90°
	b = 5.066(6) Å β= 98.53(3)°
	c = 17.43(2) Å γ= 90°
Volume	635.2(13) Å3
Z, Z'	2,1
Density (calculated)	1.152 Mg/m^3
Wavelength	0.71073 Å
Temperature	100(2) K
F(000)	240
Absorption coefficient	0.075 mm^{-1}
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9970 and 0.9613
Theta range for data collection	2.36 to 22.99°
Reflections collected	4287
Independent reflections	1000 [R (int) = 0.1127]
Data / restraints / parameters	1000 / 1 / 150
wR(F^2 all data)	wR2 = 0.1720
R(*F* obsd data)	R1 = 0.0710
Goodness-of-fit on F^2	1.051
Observed data [I > 2σ (I)]	730
Absolute structure parameter	-3(5)
Largest and mean shift / s.u.	0.000 and 0.000
Largest diff. peak and hole	0.227 and -0.257 e/Å3

Table S3. Sequence of large-ribosomal-subunit ITSI region of the rDNA gene

ATTGNTCCTCCNGATCGAGGGTCACCTGATGAAATAGATTGGTTGCTTTTCAGCGTCGGCCAG
CAGCCGGCCGGGCCTACAAGAGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGT
GCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGGGGGGGACGACGACCCAACACACAAGCCGT
GCTTGAGGGCAGCAATGACGCTCGGACAGGCATACCCCCCGGAATACCAGGGGGTGCAATGT
GCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAATTATCGCATTTCGCTGCG
TTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATACAAT
CGACTCAGACGACAAAACTTCAGACAGTGTTCACGTTGGTGTCTCCGGCGAGCGCTGTGCCAC
CCCTAAGGAAGGGGGGCGGGCTCGCCGAAGCAACAAGGTACGGTATACACGGGTGGGAGGTT
GGGCCCCGAGGGACCCTCACTCAGTAATGATCCTTCCGCAGGTCNCCNTTACGGAAGGGNNN
NNNNNNN

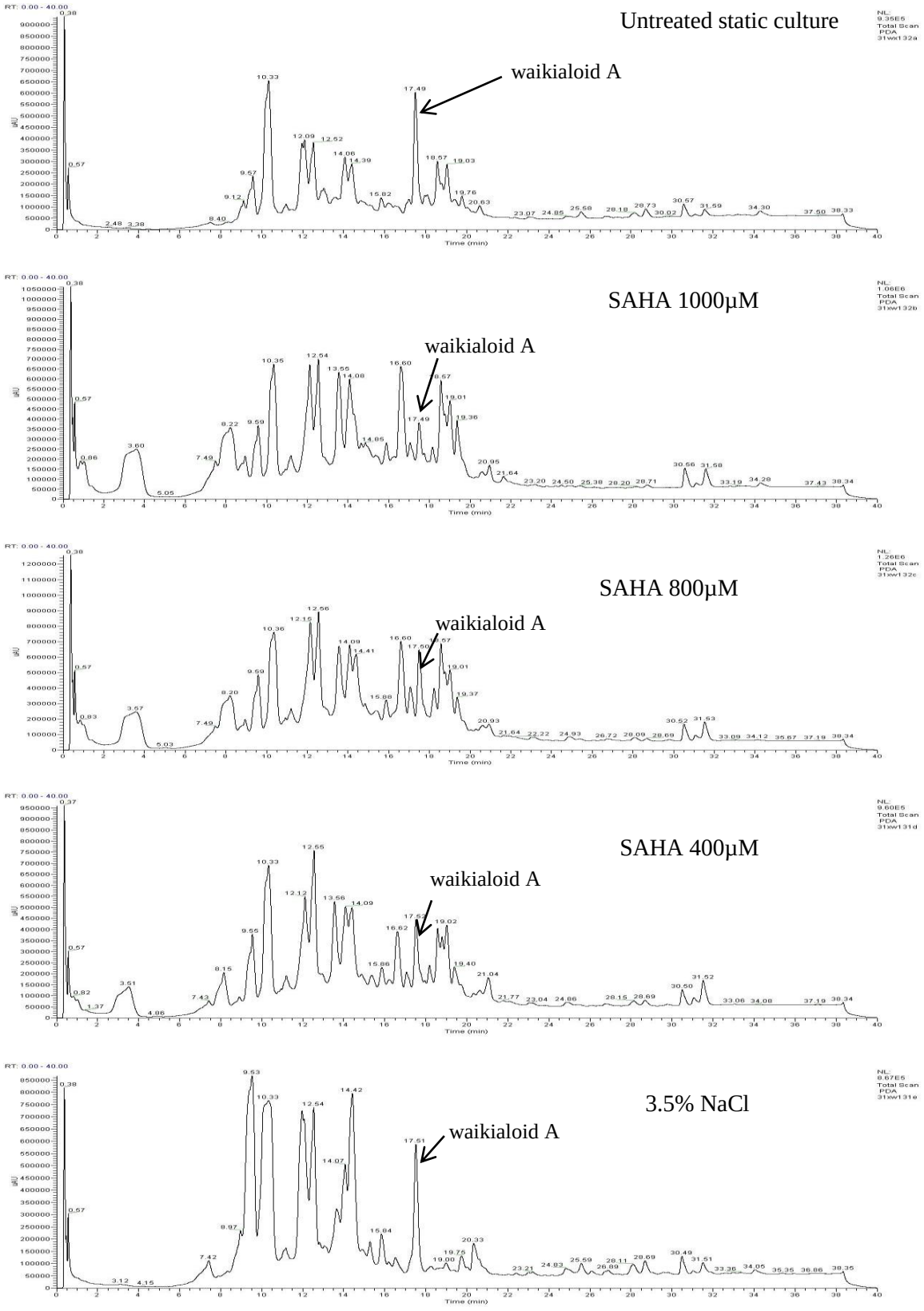


Figure S1. LC-MS profiles of extracts prepared for the Hawaiian *Aspergillus* sp. isolate.

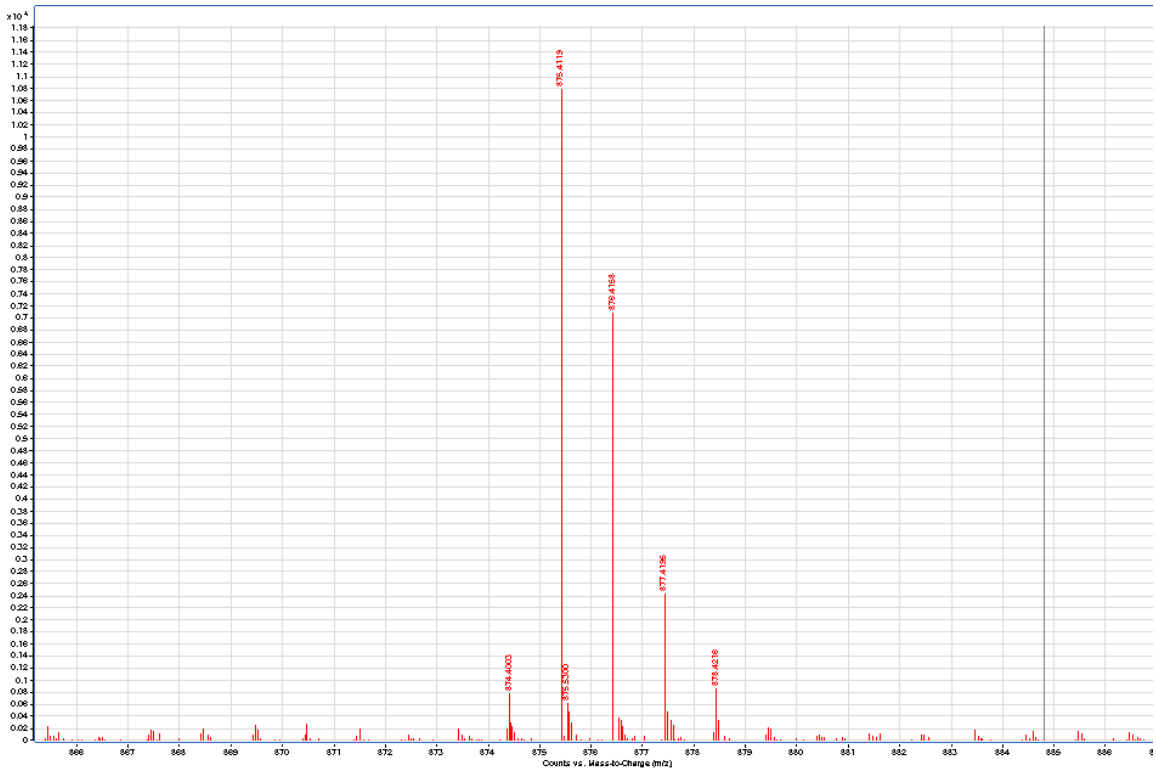


Figure S2. HRESIMS of waikialoid A (**1**).

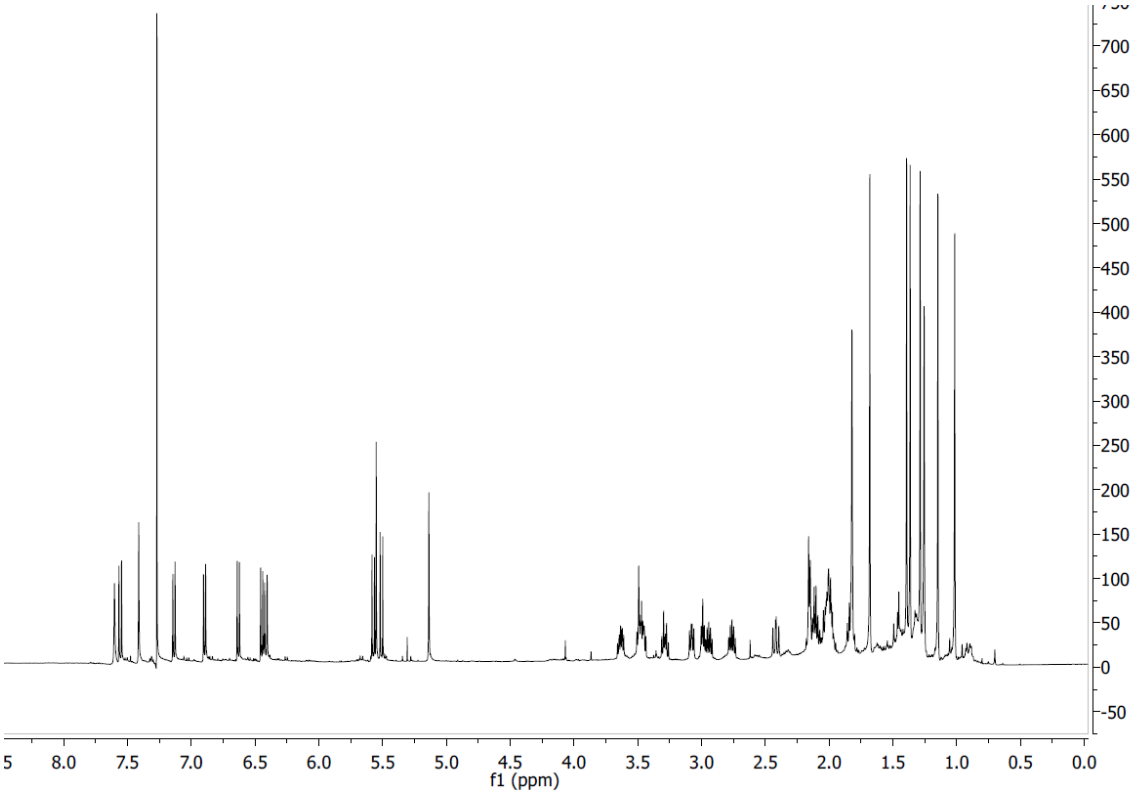


Figure S3. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid A (**1**).

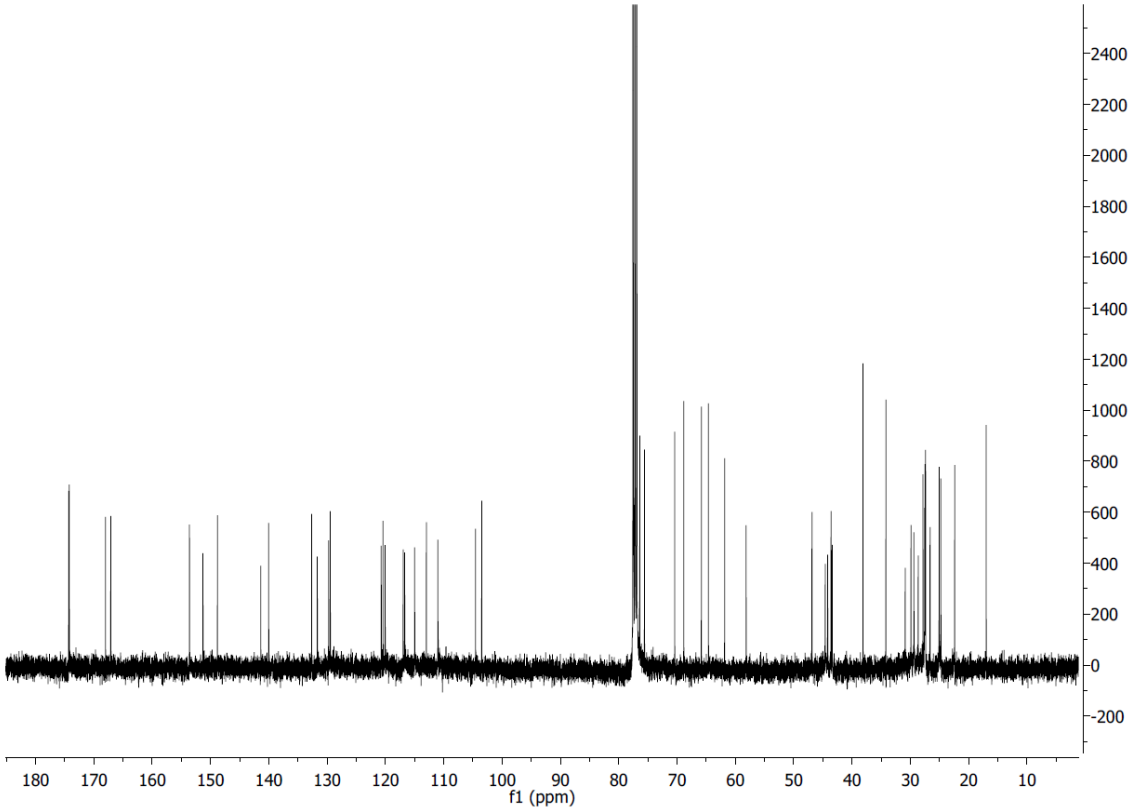


Figure S4. ^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of waikialoid A (**1**).

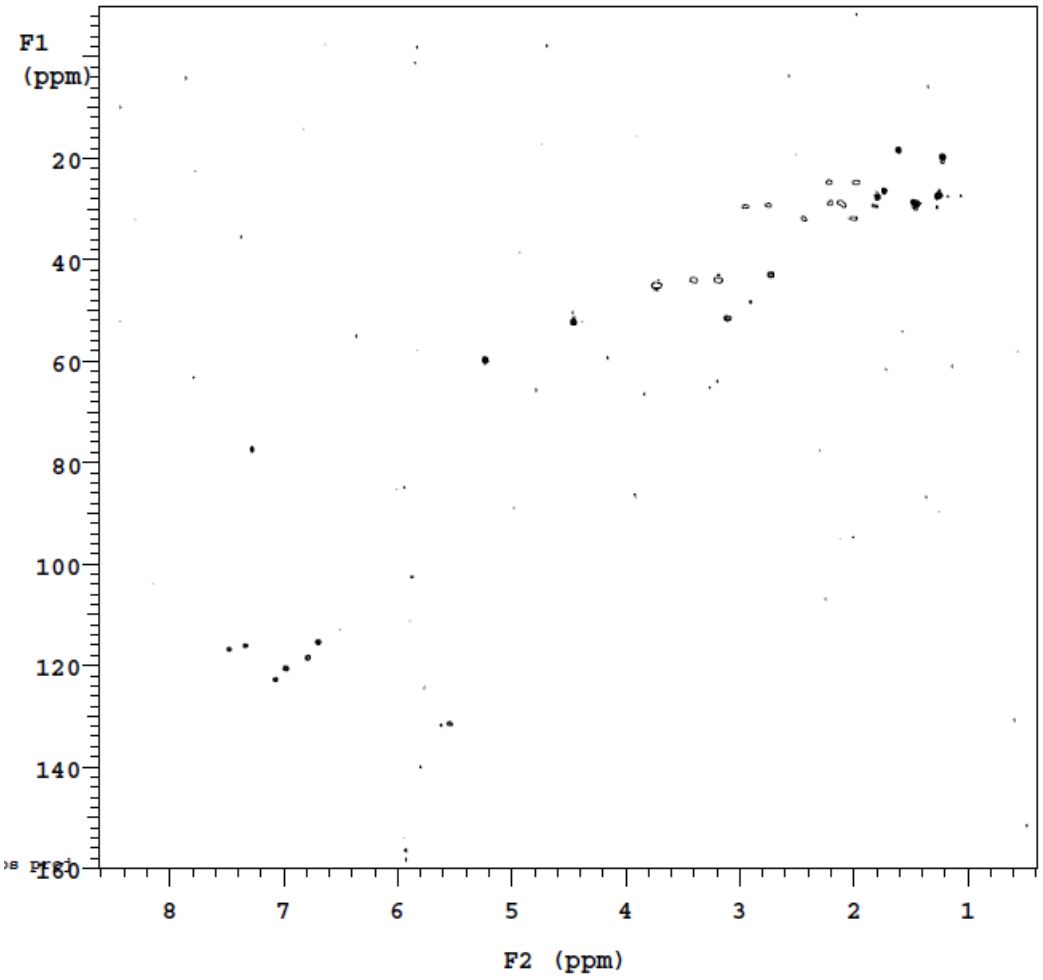


Figure S5. HSQC-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid A (**1**).

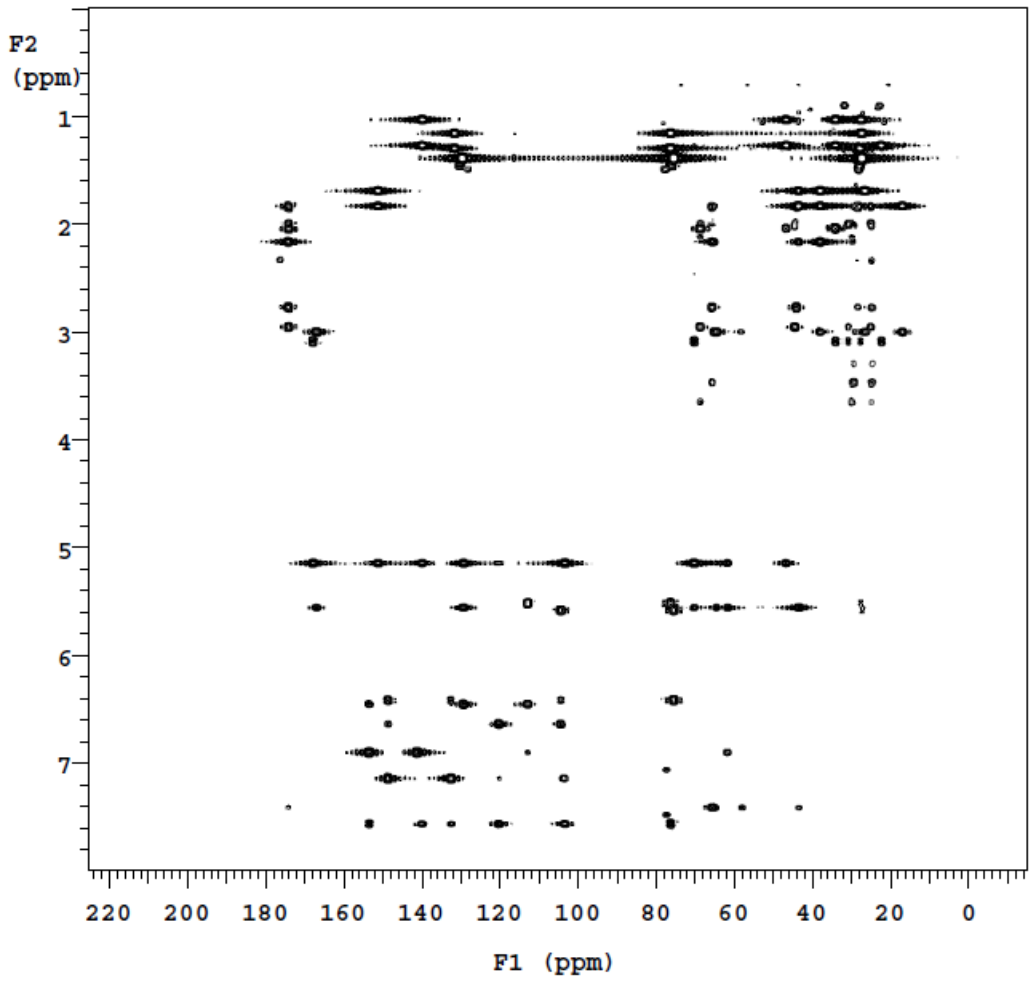


Figure S6. HMBC-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid A (**1**).

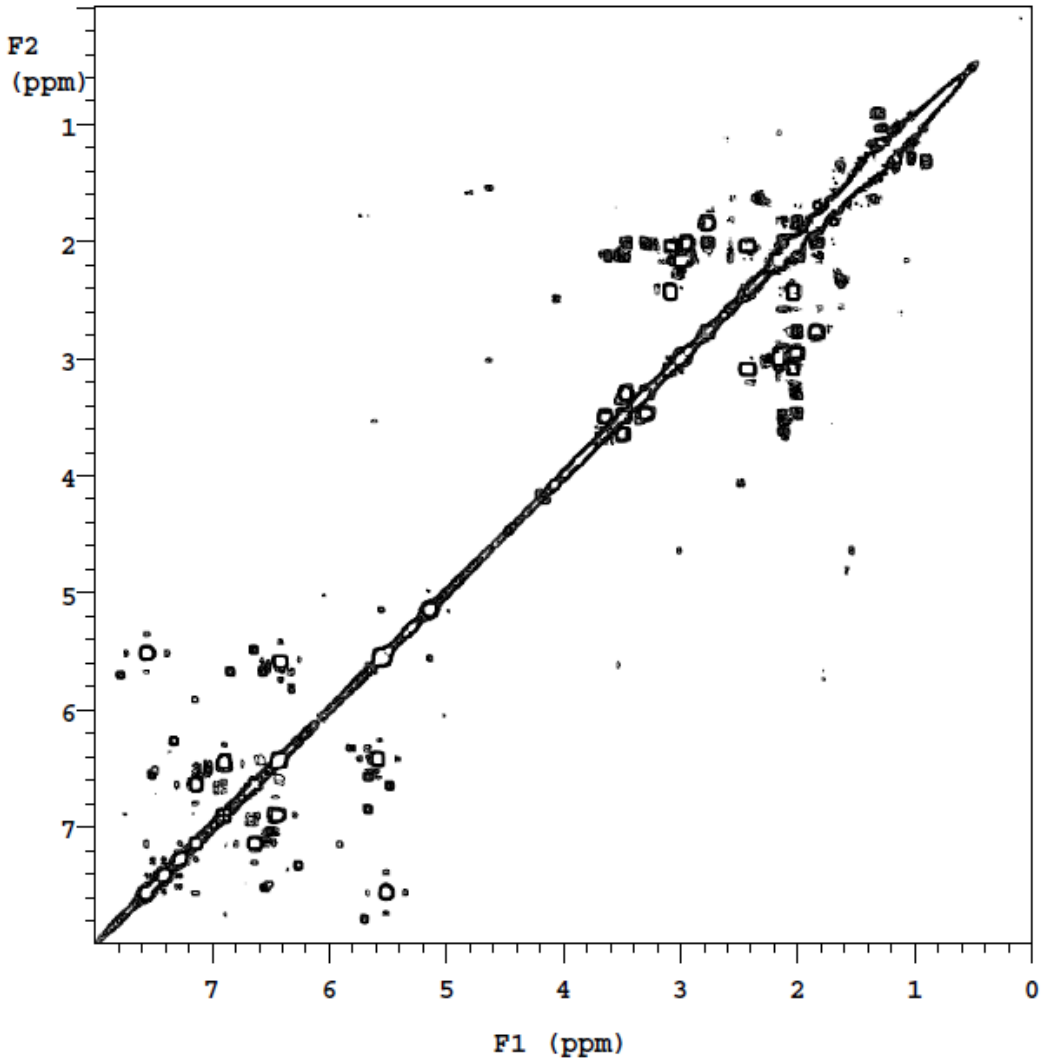


Figure S7. COSY-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid A (**1**).

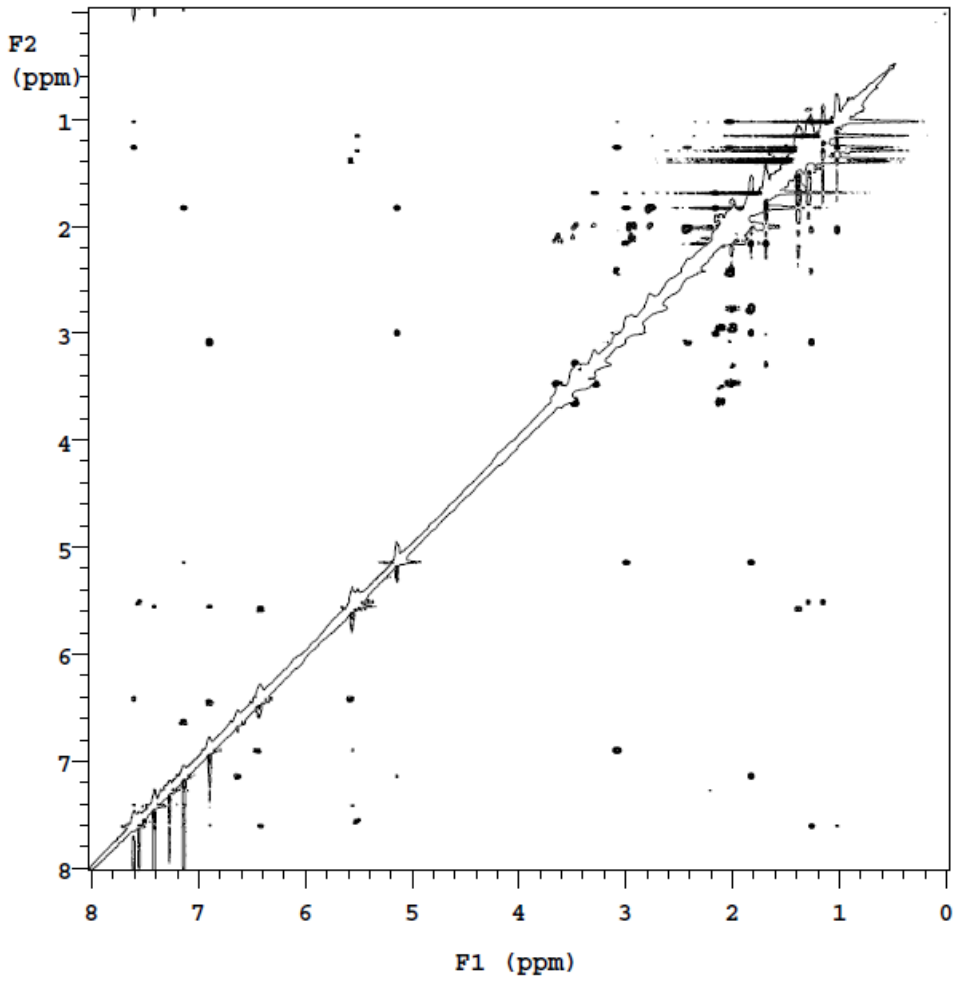


Figure S8. NOESY-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid A (**1**).

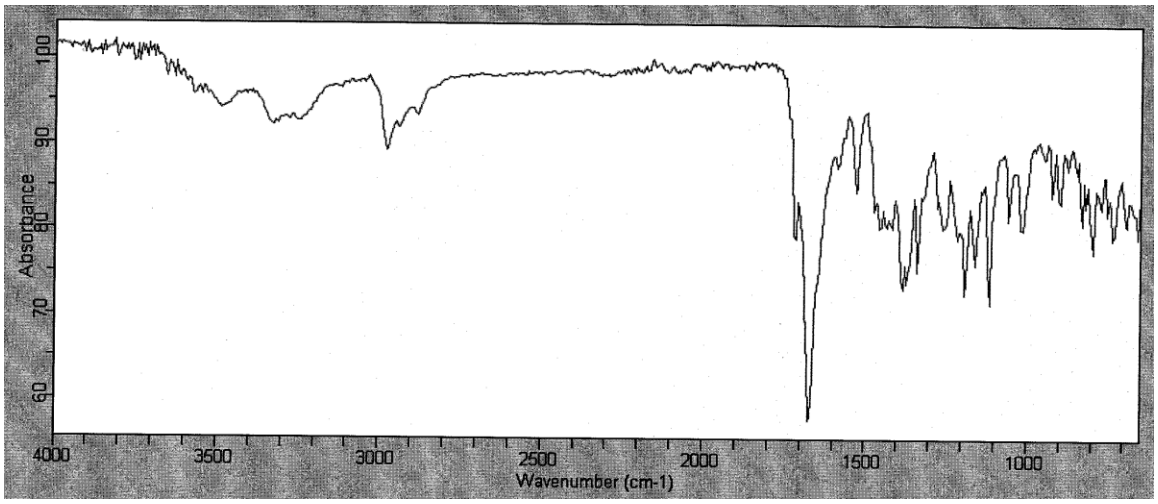


Figure S9. FTIR spectrum of waikialoid A (**1**).

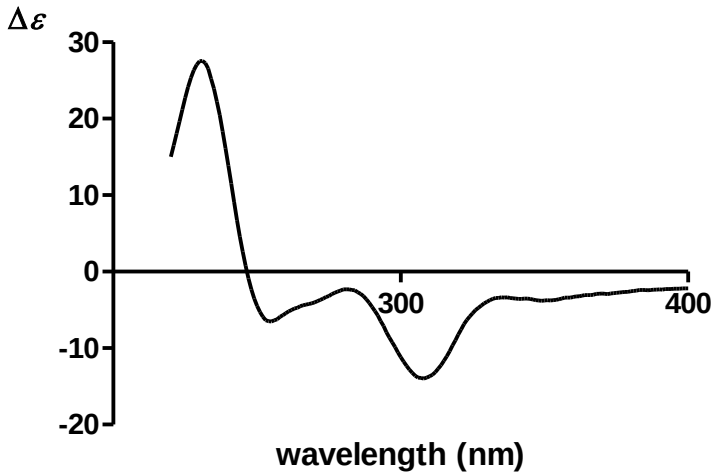


Figure S10. CD spectrum of waikialoid A **(1).**

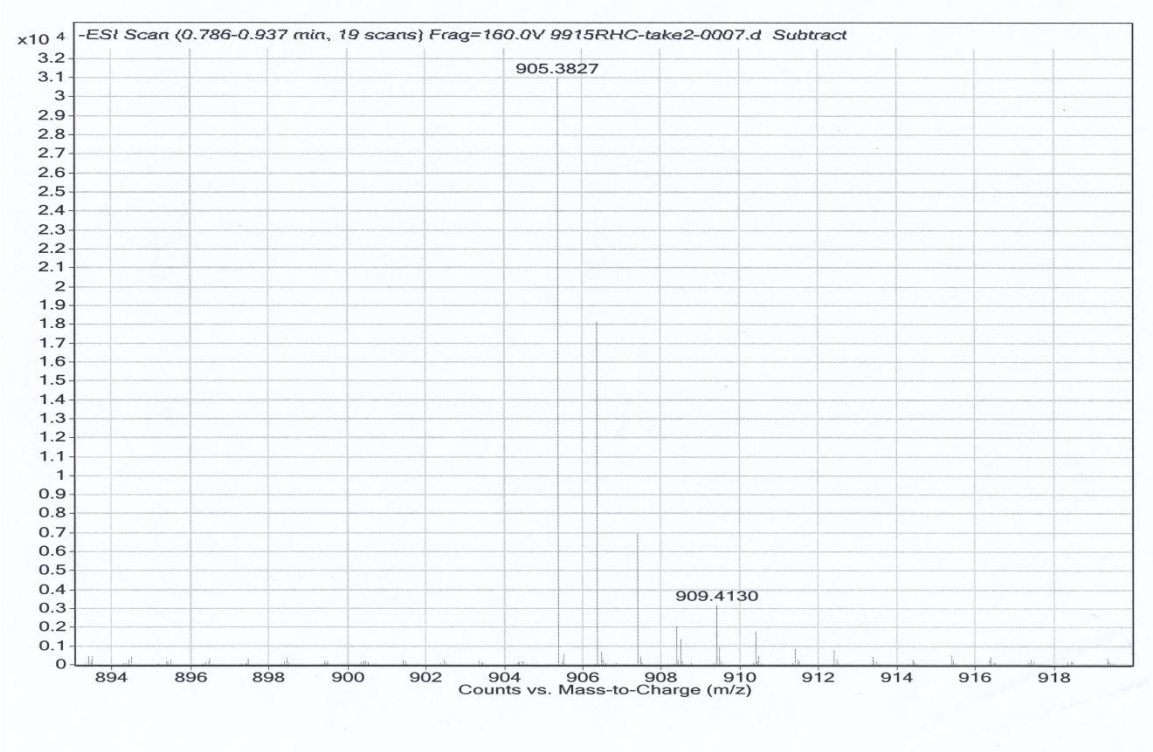


Figure S11. HRESIMS of waikialoid B (**14**).

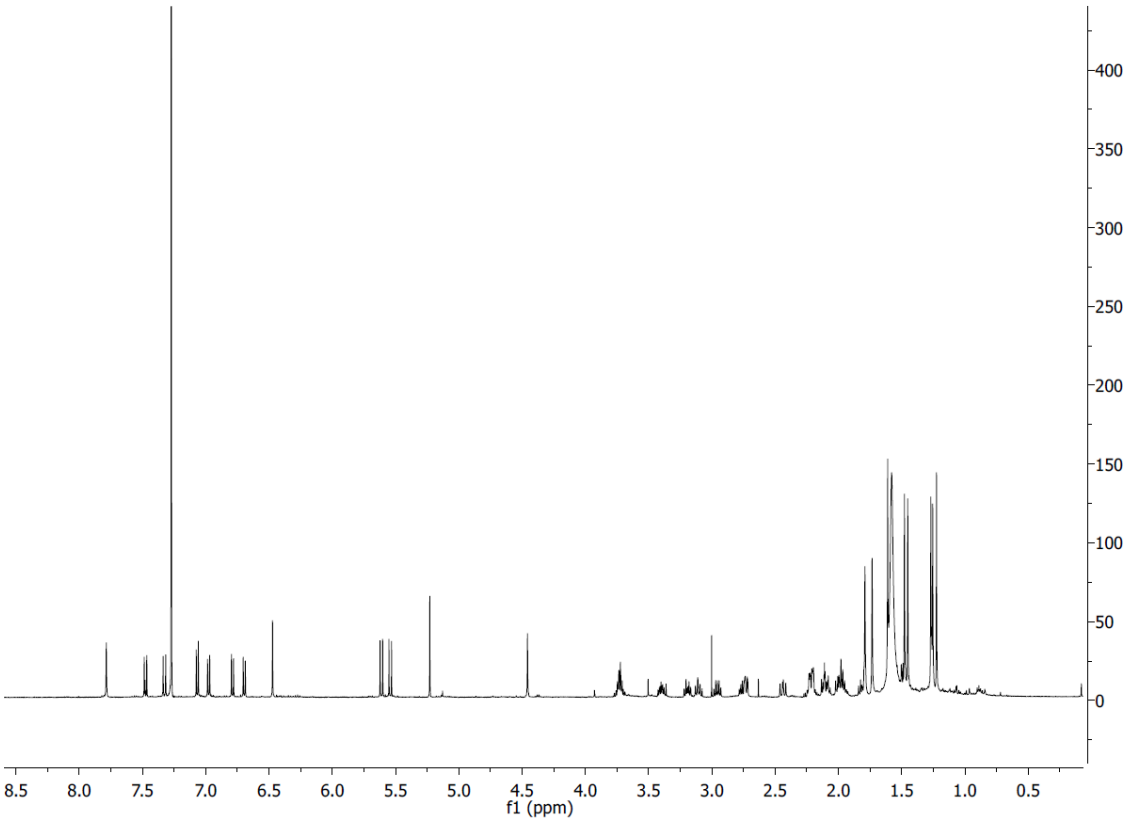


Figure S12. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid B (**14**).

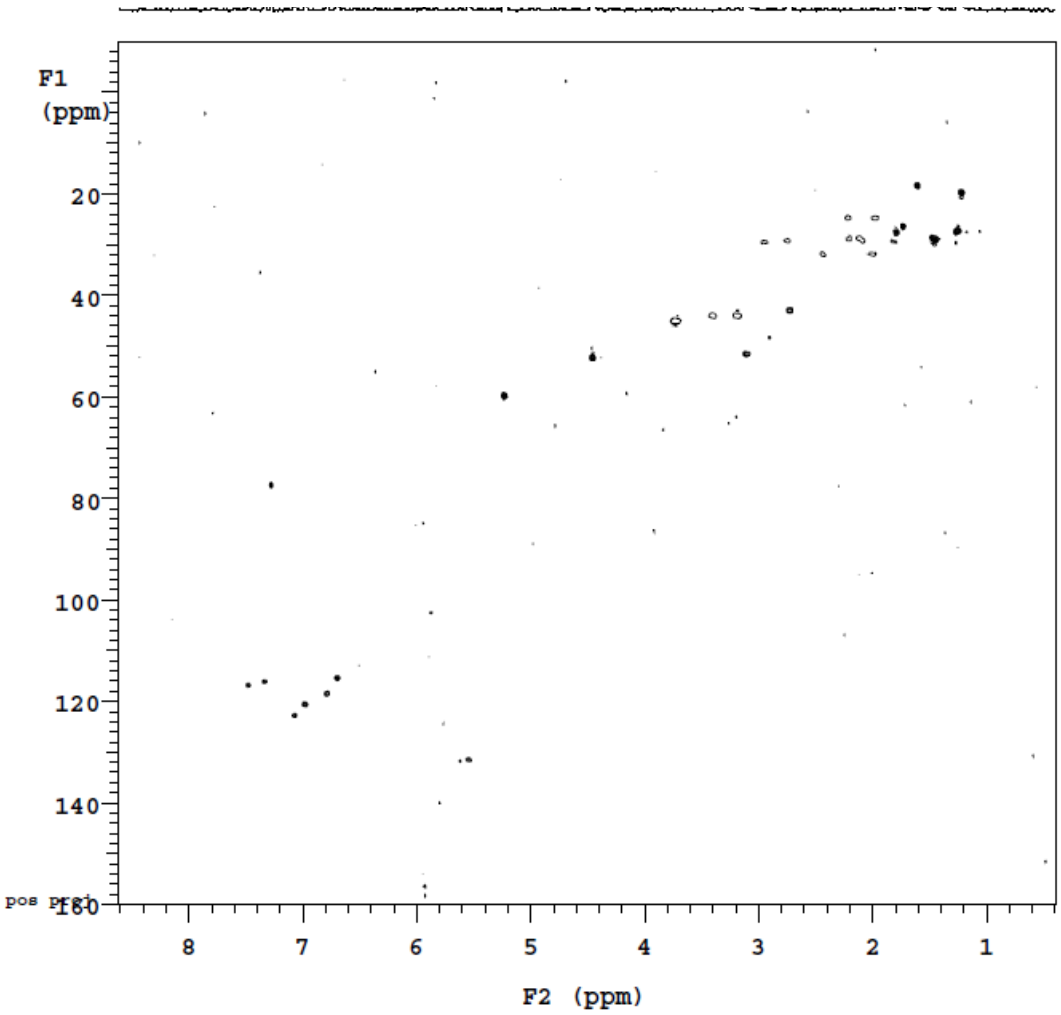


Figure S13. HSQC-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid B (**14**).

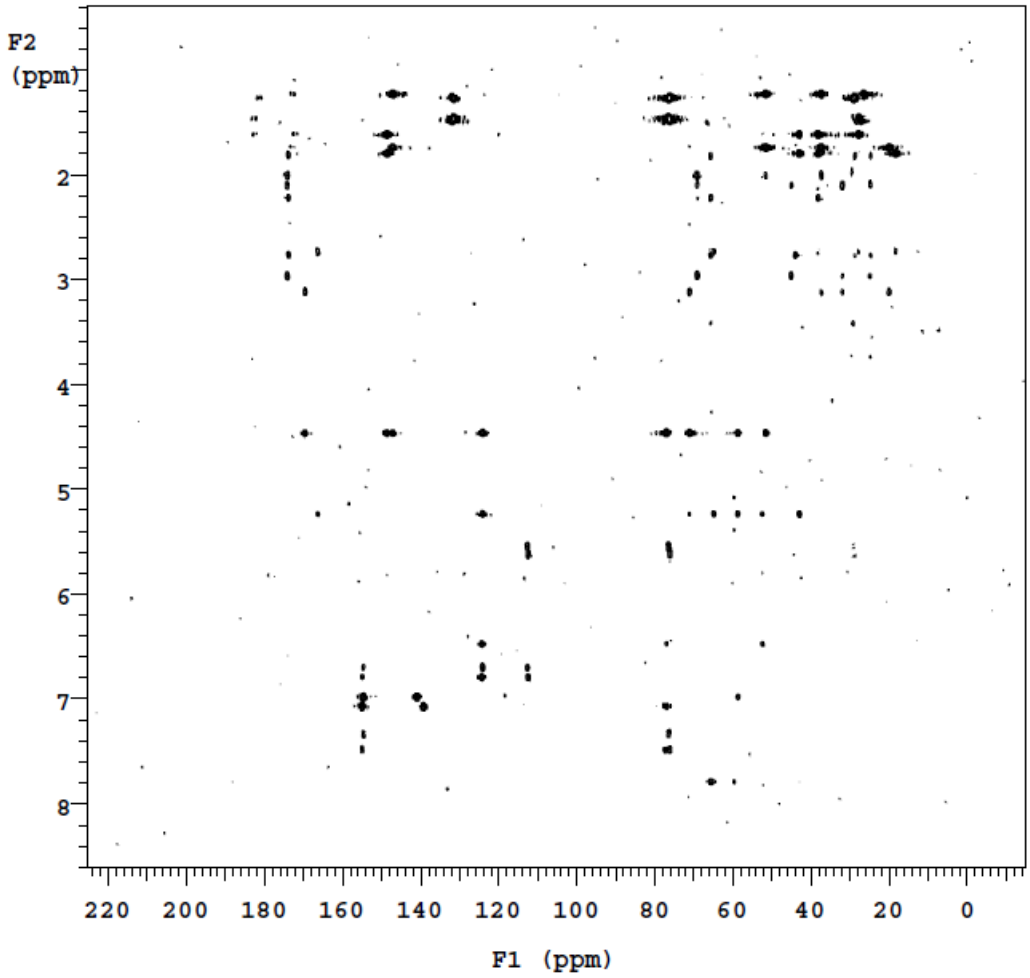


Figure S14. HMBC-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid B (**14**).

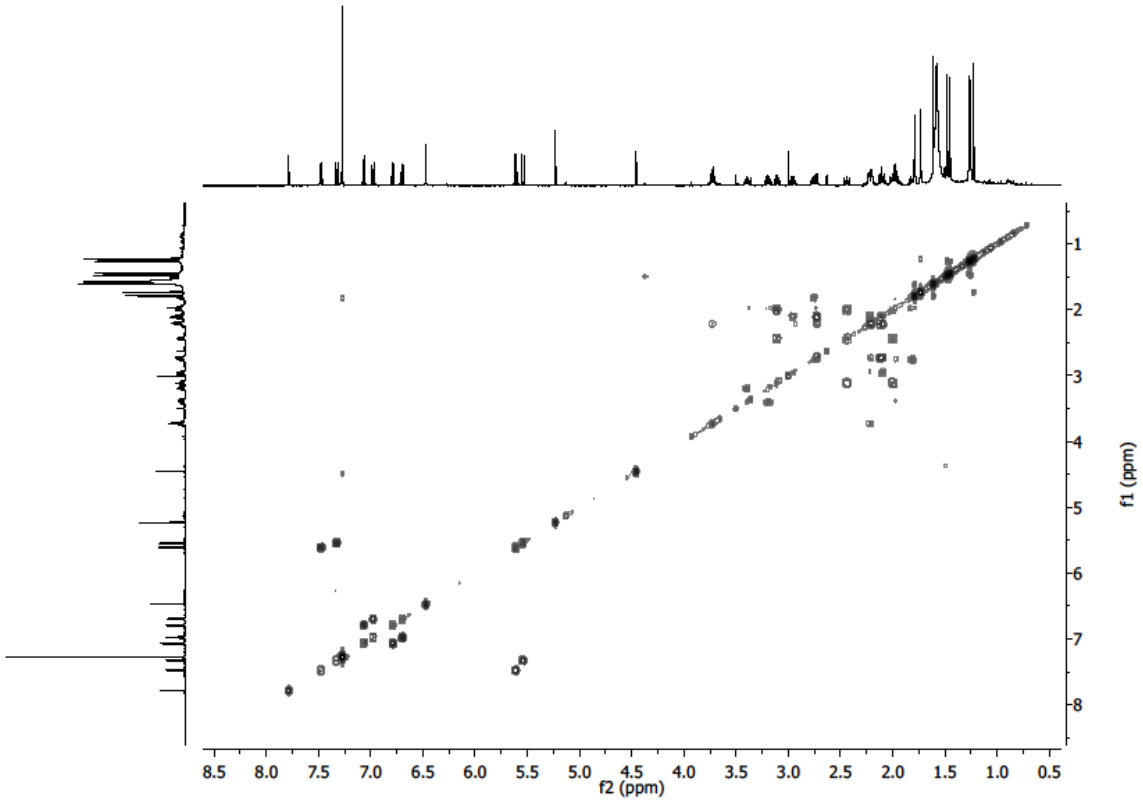


Figure S15. COSY-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid B (**14**).

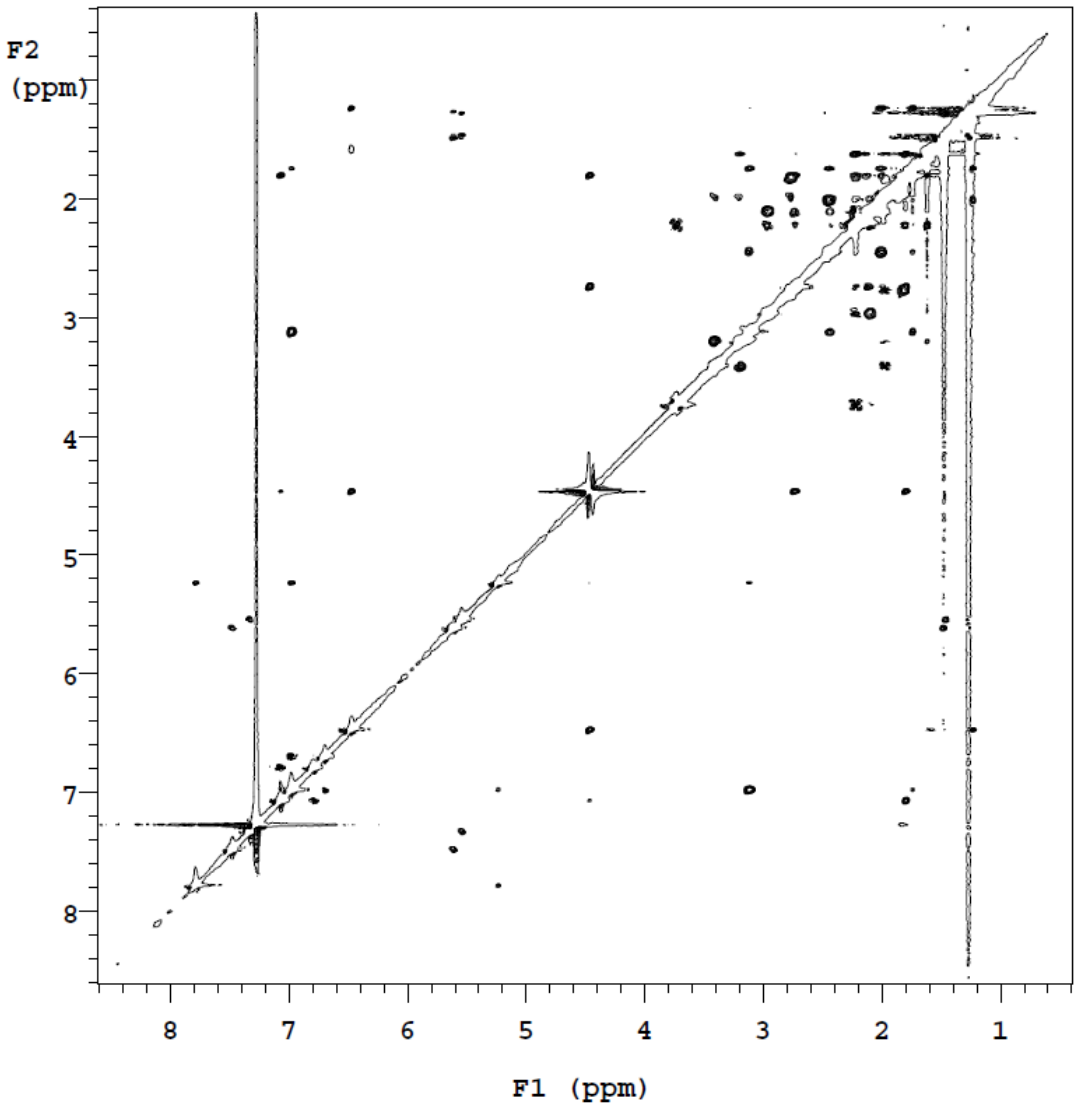


Figure S16. ROESY-NMR spectrum (500 MHz, $CDCl_3$) of waikialoid B (**14**).

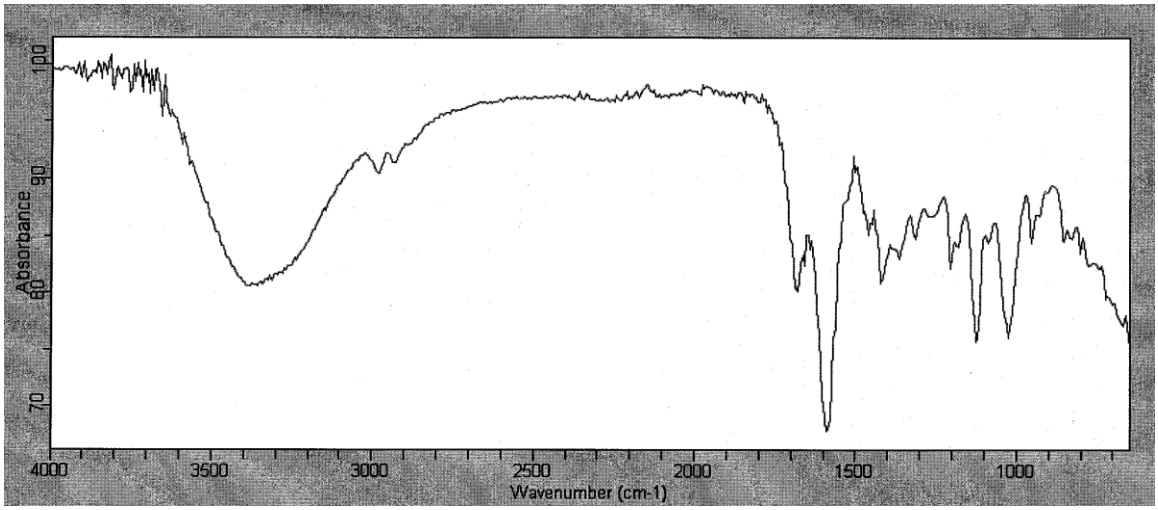


Figure S17. FTIR spectrum of waikialoid B (**14**).

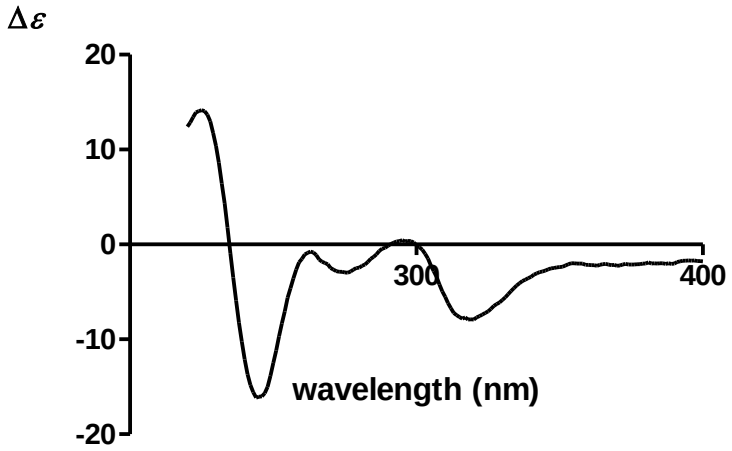


Figure S18. CD spectrum of waikialoid B (**14**).

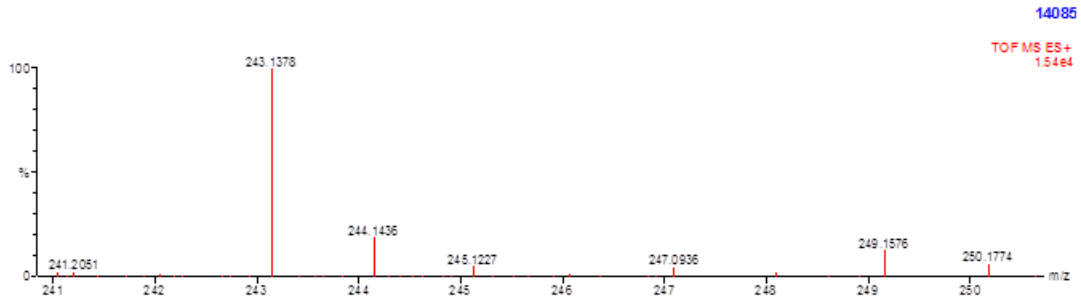


Figure S19. HRESIMS of waikialide A **(15).**

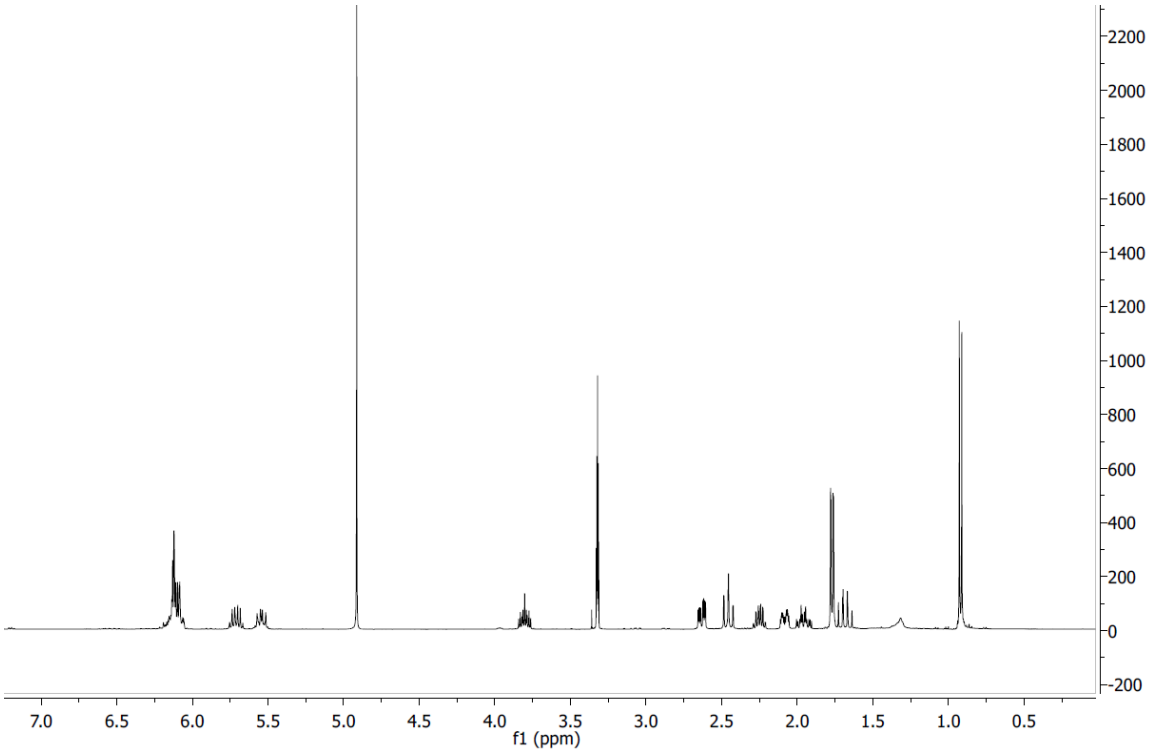


Figure S20. ^{1}H-NMR spectrum (400 MHz, CD_3OD) waikialide A **(15).**

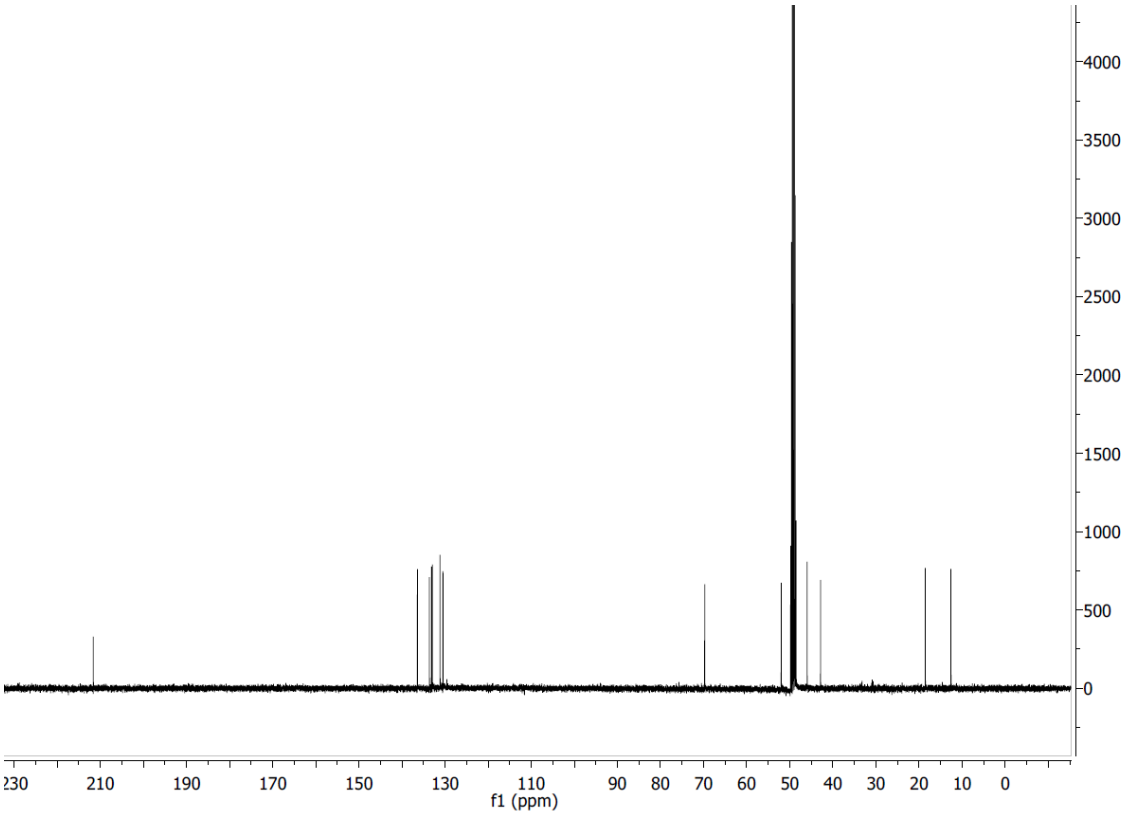


Figure S21. ^{13}C-NMR spectrum (100 MHz, CD_3OD) waikialide A **(15).**

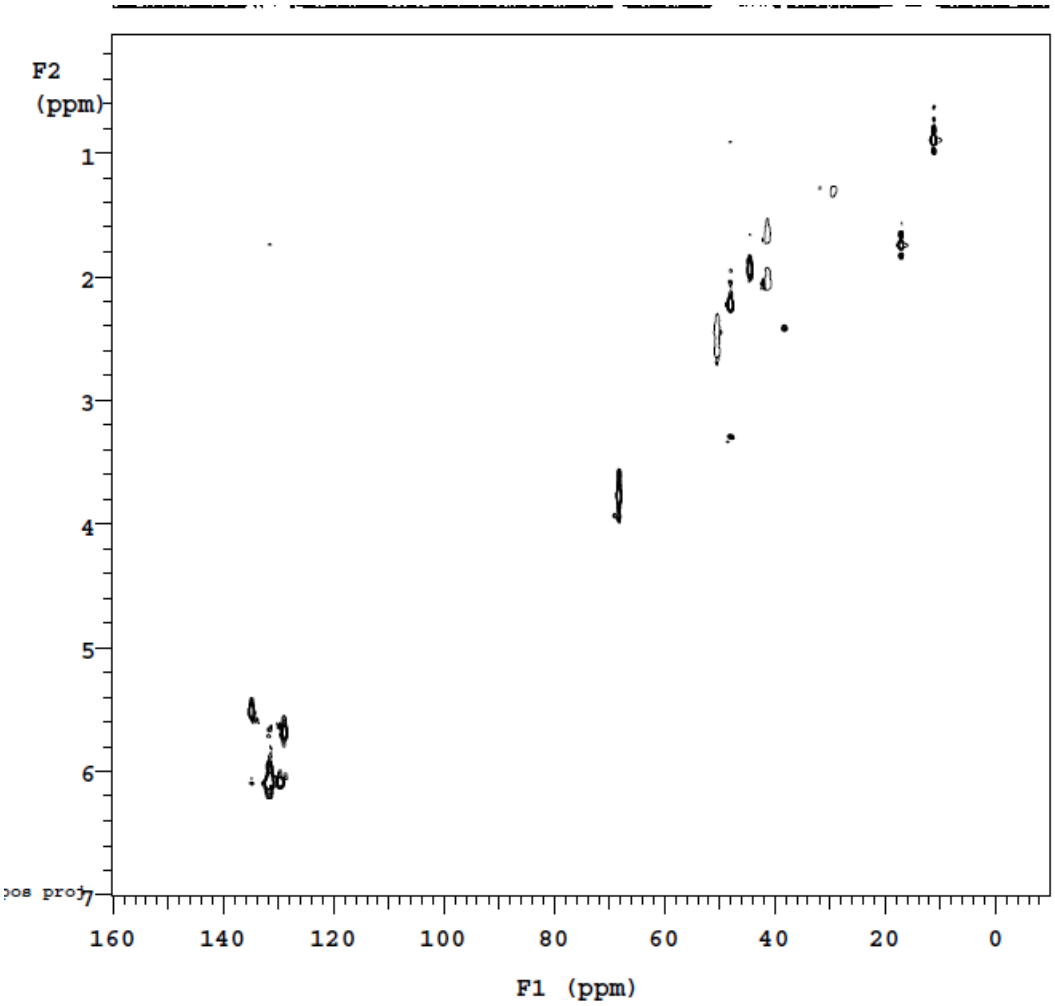


Figure S22. HSQC-NMR spectrum (400 MHz, CD_3OD) waikialide A **(15).**

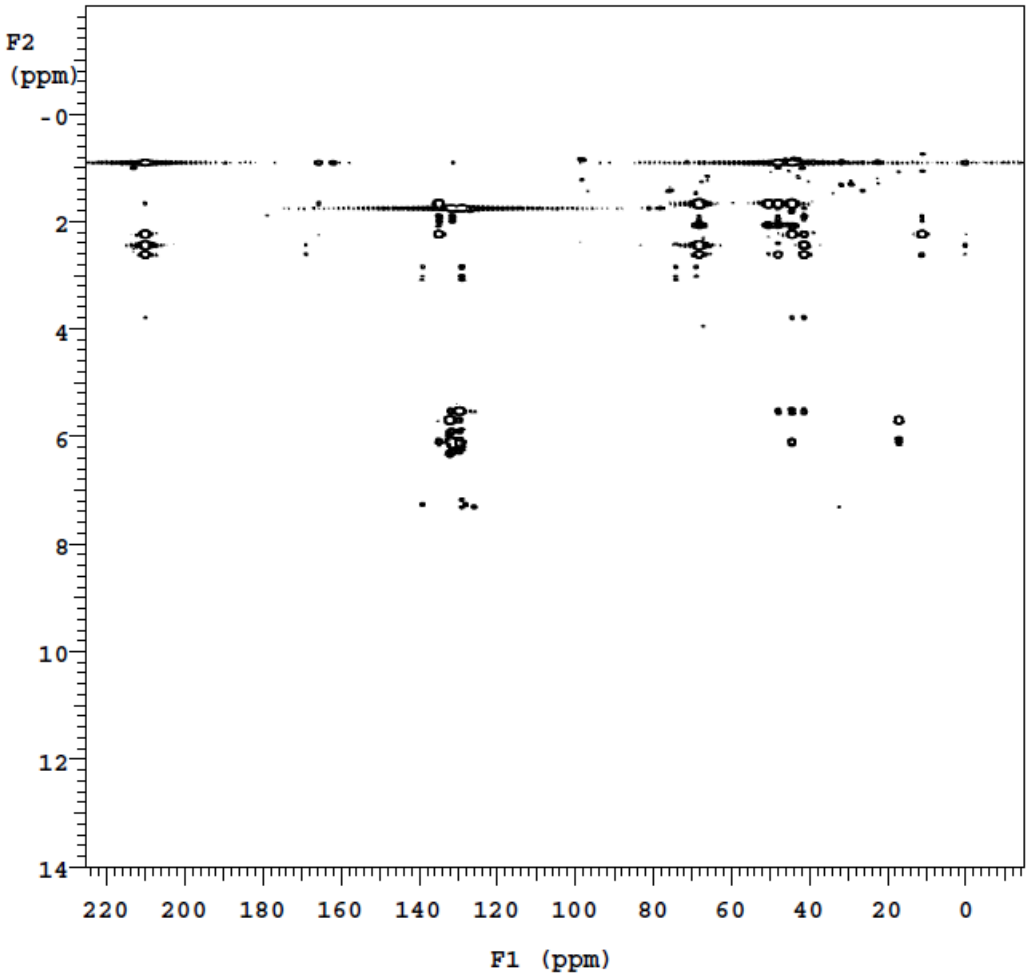


Figure S23. HMBC-NMR spectrum (500 MHz, CD_3OD) waikialide A **(15).**

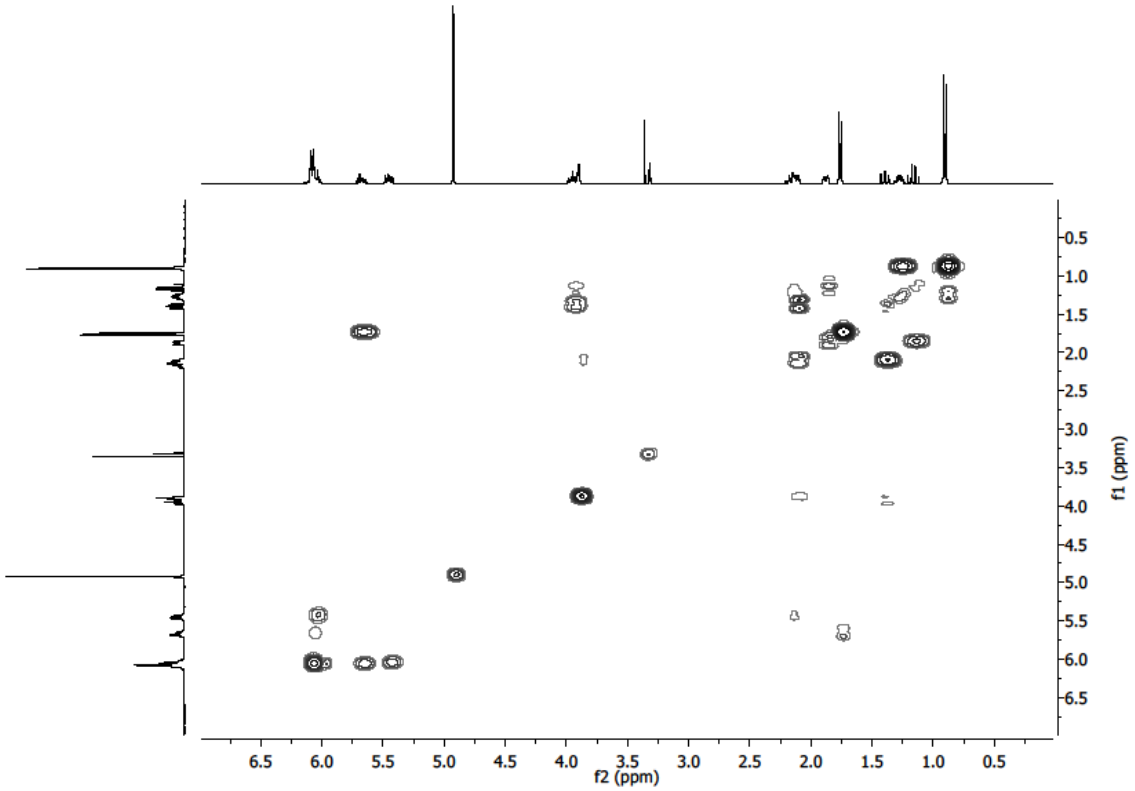


Figure S24. COSY-NMR spectrum (400 MHz, CD_3OD) of waikialide A **(15).**

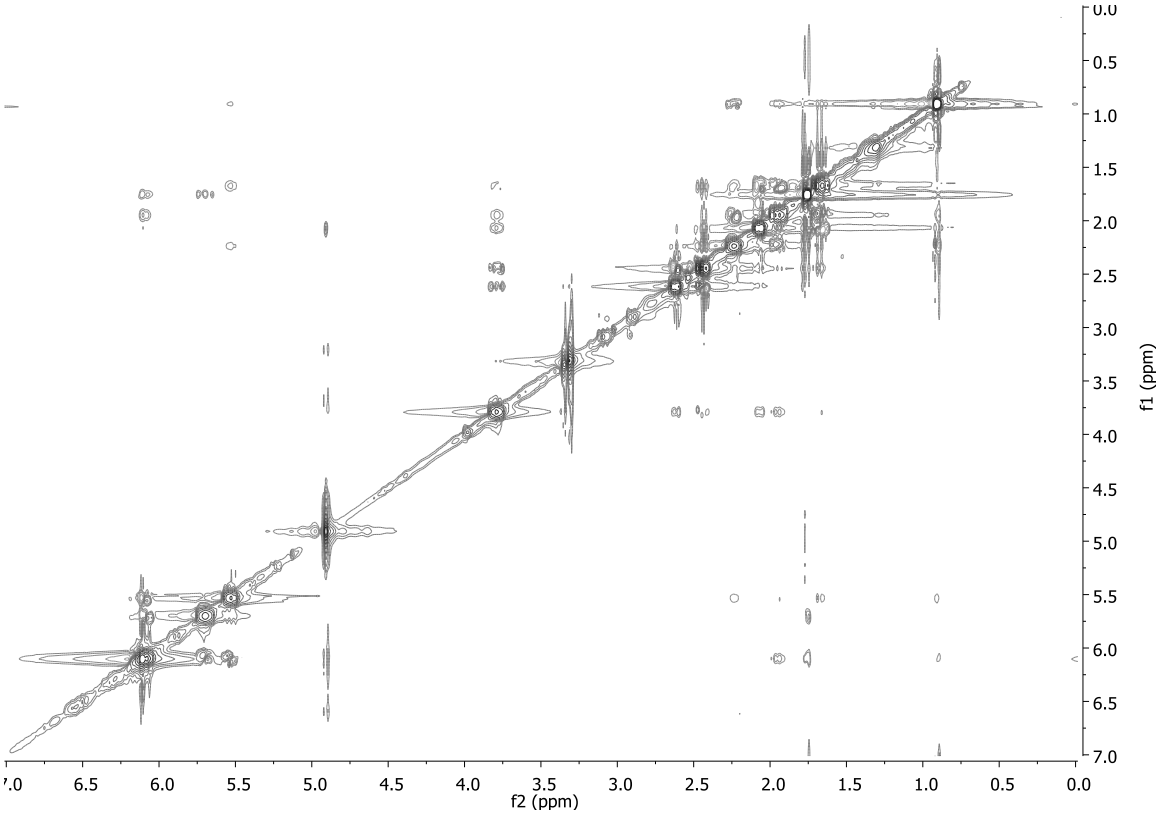


Figure S25. NOESY-NMR spectrum (400 MHz, CD_3OD) of waikialide A **(15).**

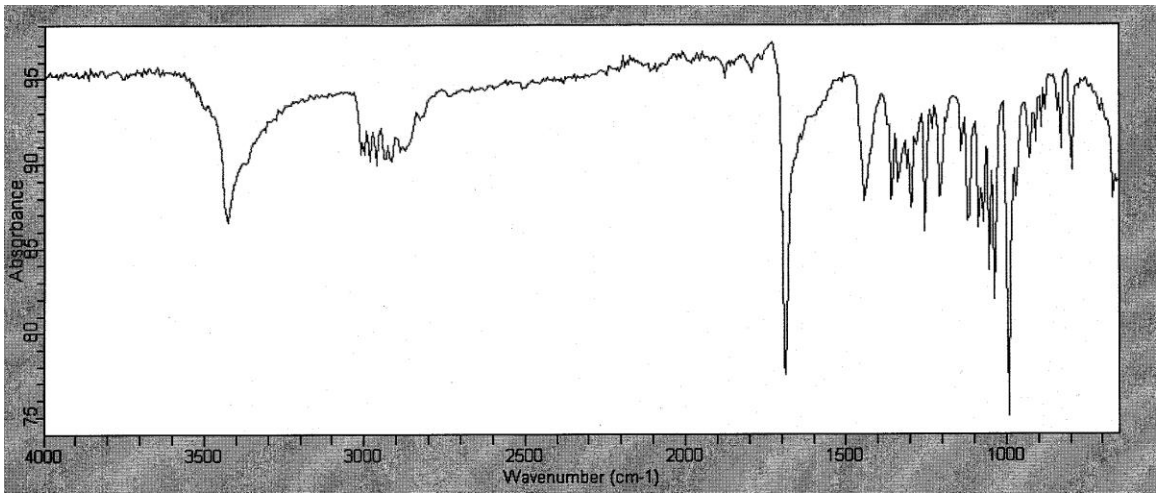


Figure S26. FTIR spectrum of waikialide A **(15).**

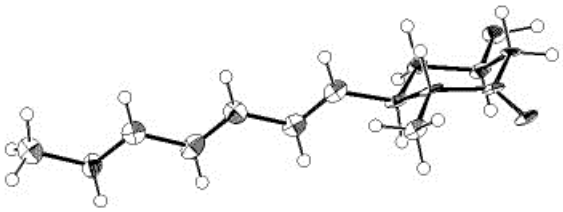


Figure S27. ORTEP structure for **15** generated from the X-ray diffraction data.

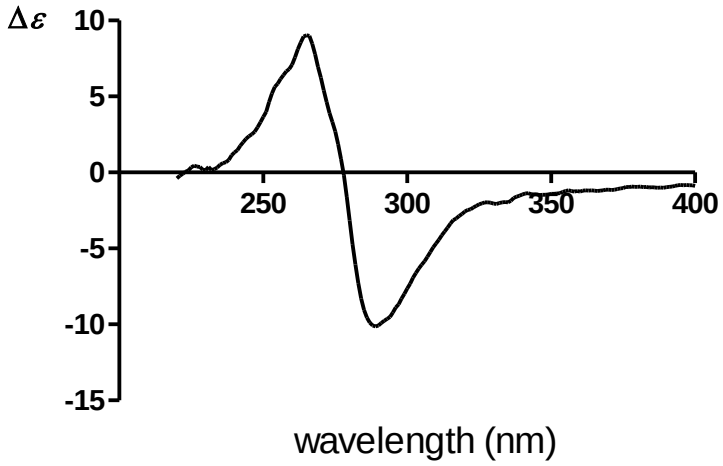


Figure S28. CD spectrum of waikialide A **(15).**

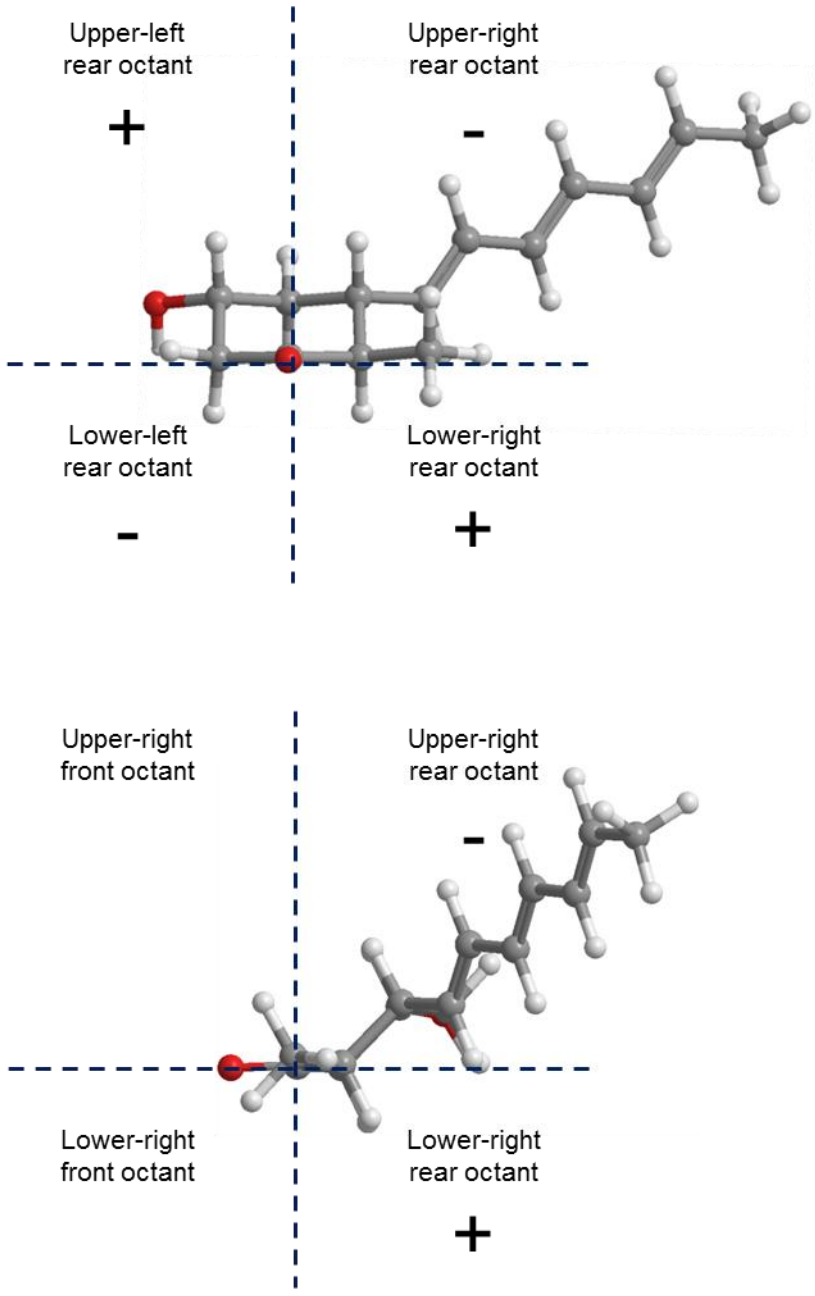


Figure S29. Octant rule applied to waikialide A **(15).**

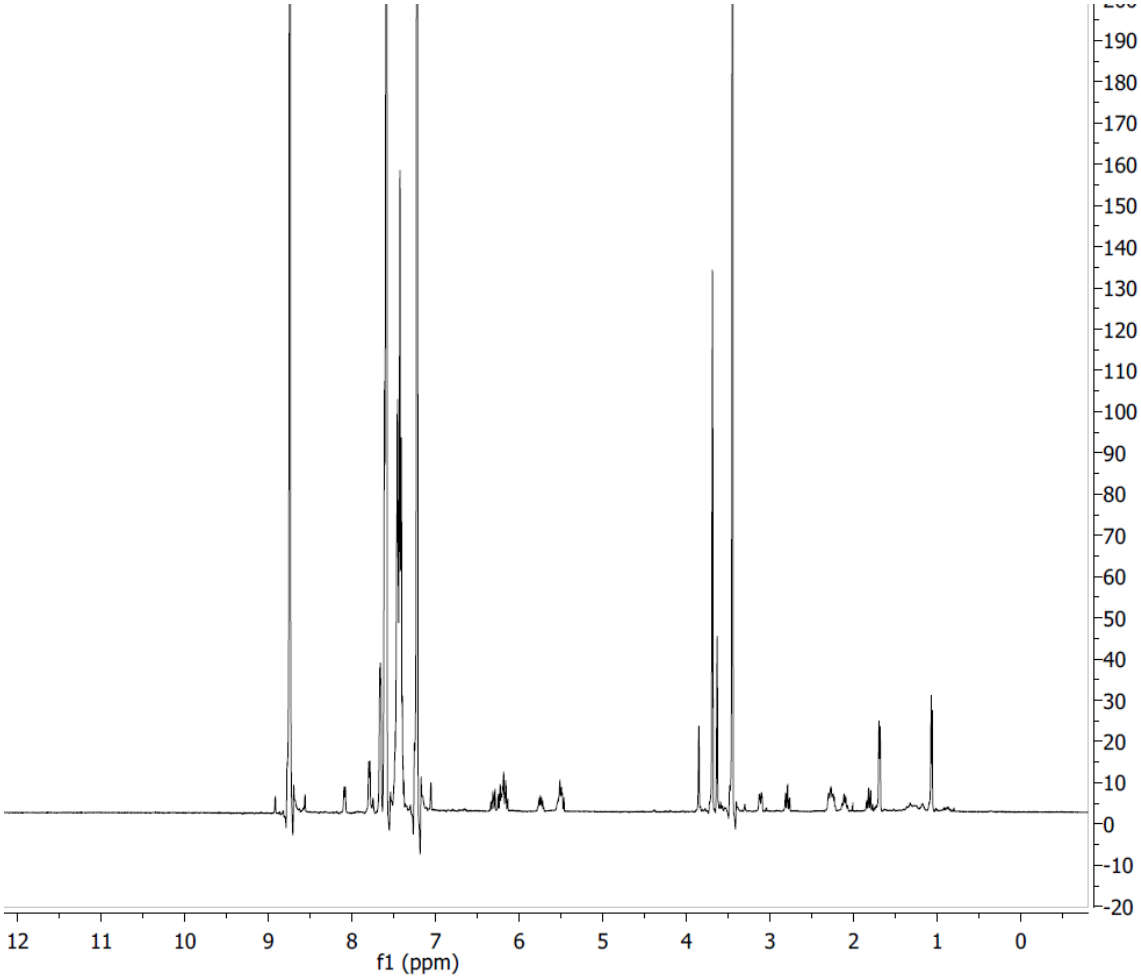


Figure S30. ^{1}H -NMR (500 MHz, pyrindine-d_5) of derivative of waikialide A (**15a**).

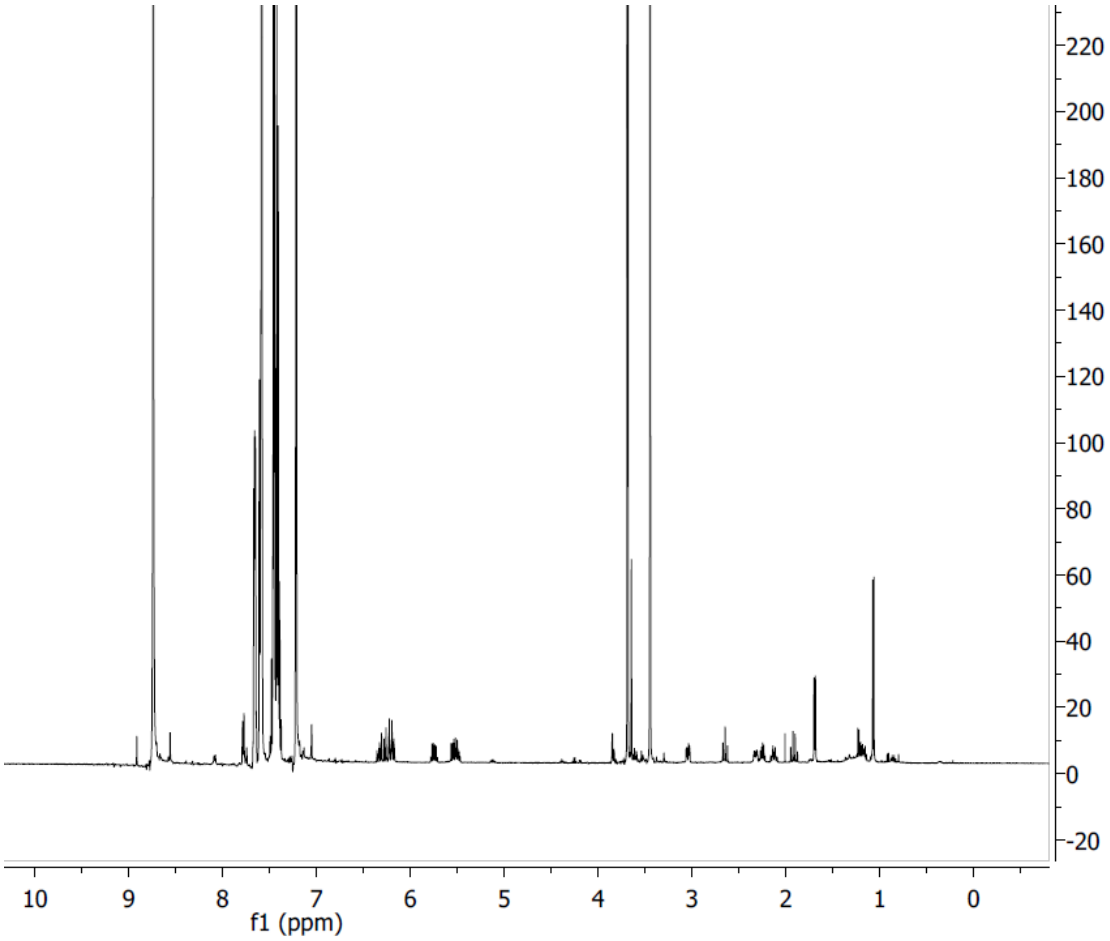


Figure S31. ^{1}H -NMR (500 MHz, pyrindine-d_5) of derivative of waikialide A (**15b**).

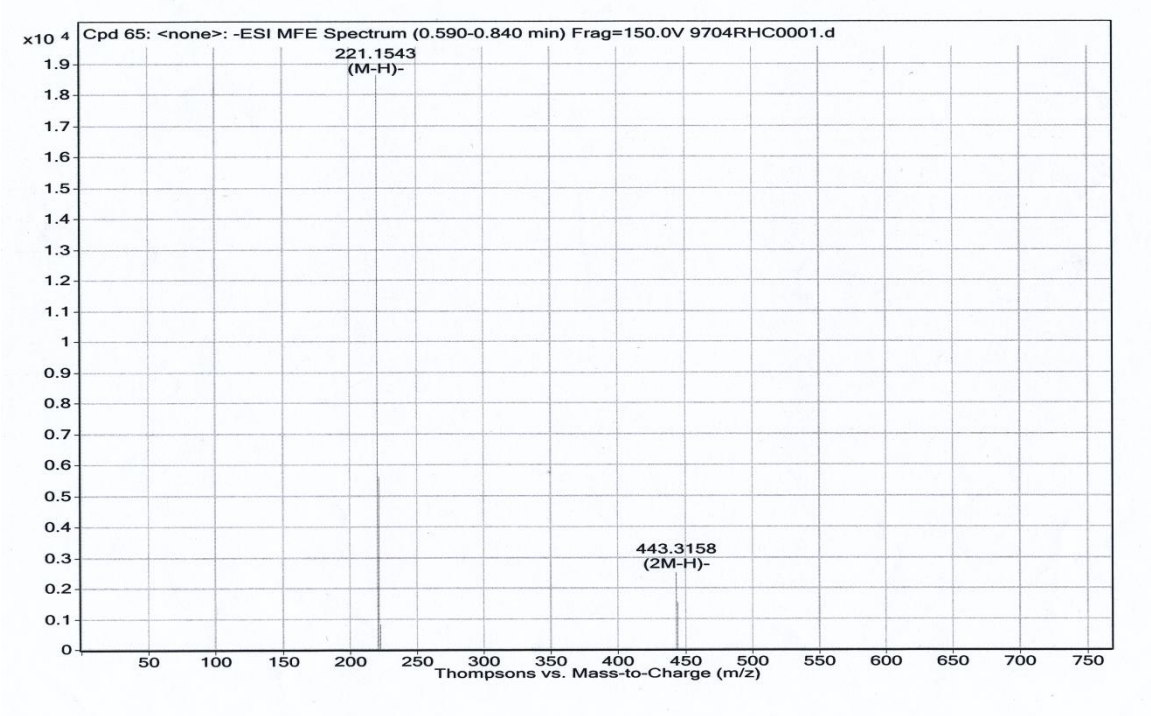


Figure S32. HRESIMS of waikialide B **(16).**

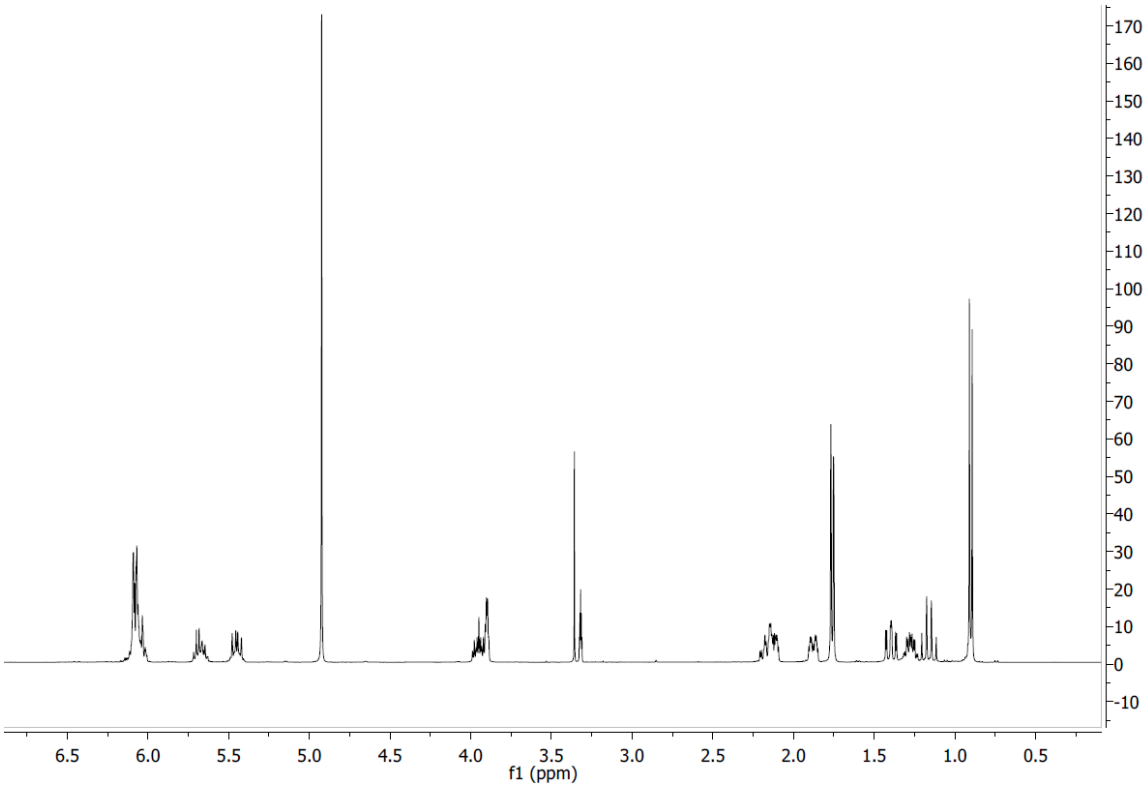


Figure S33. ^{1}H-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

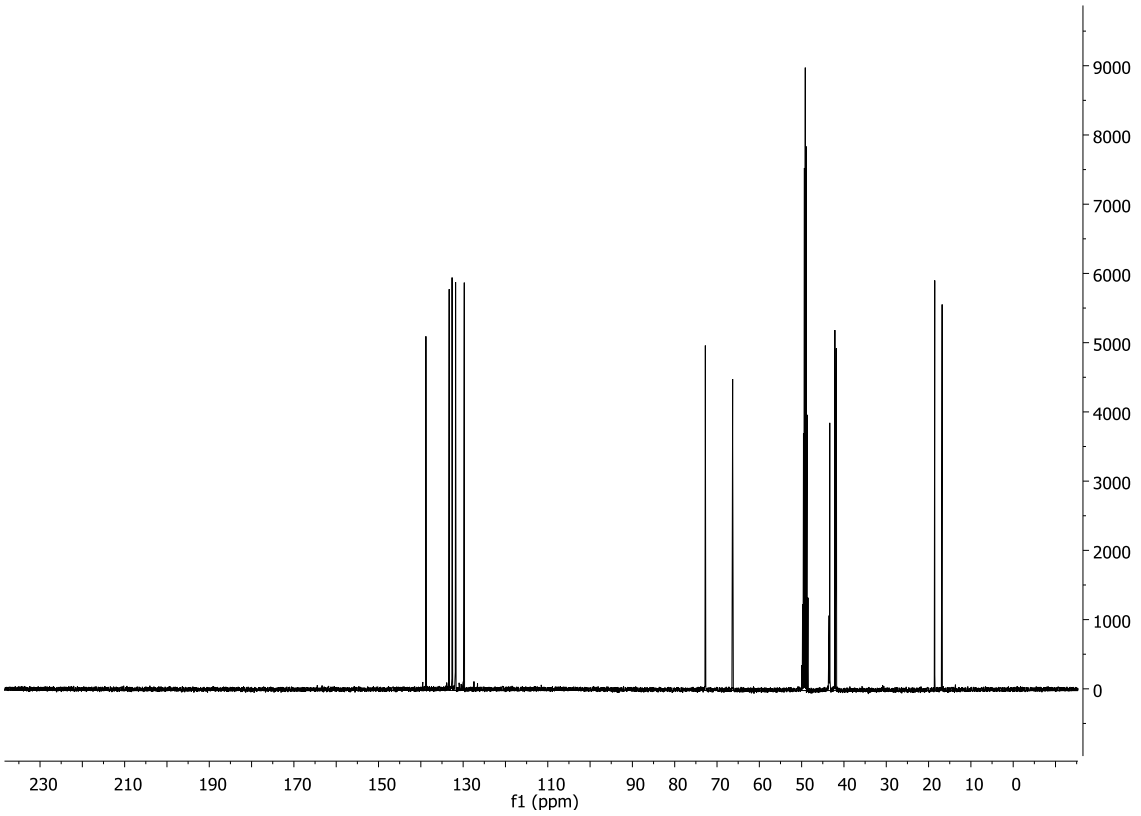


Figure S34. ^{13}C-NMR spectrum (100 MHz, CD_3OD) waikialide B **(16).**

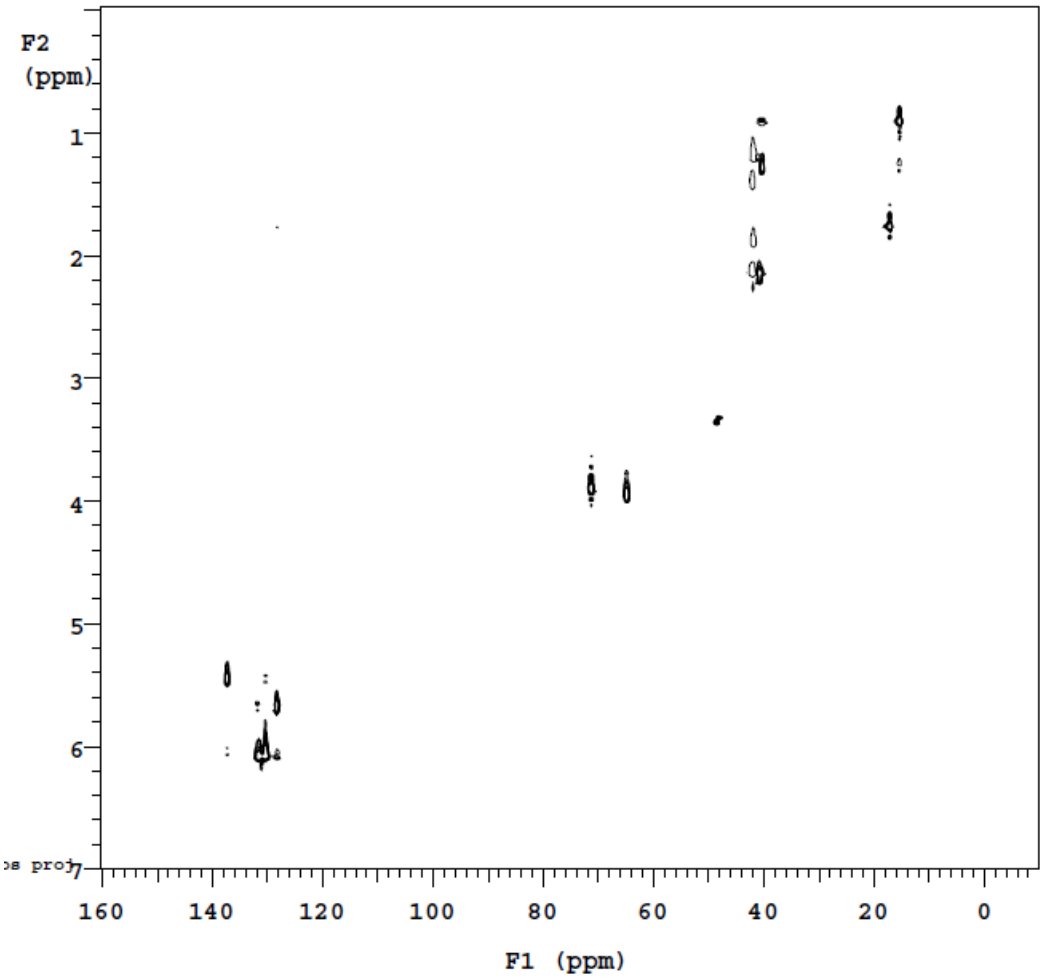


Figure S35. HSQC-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

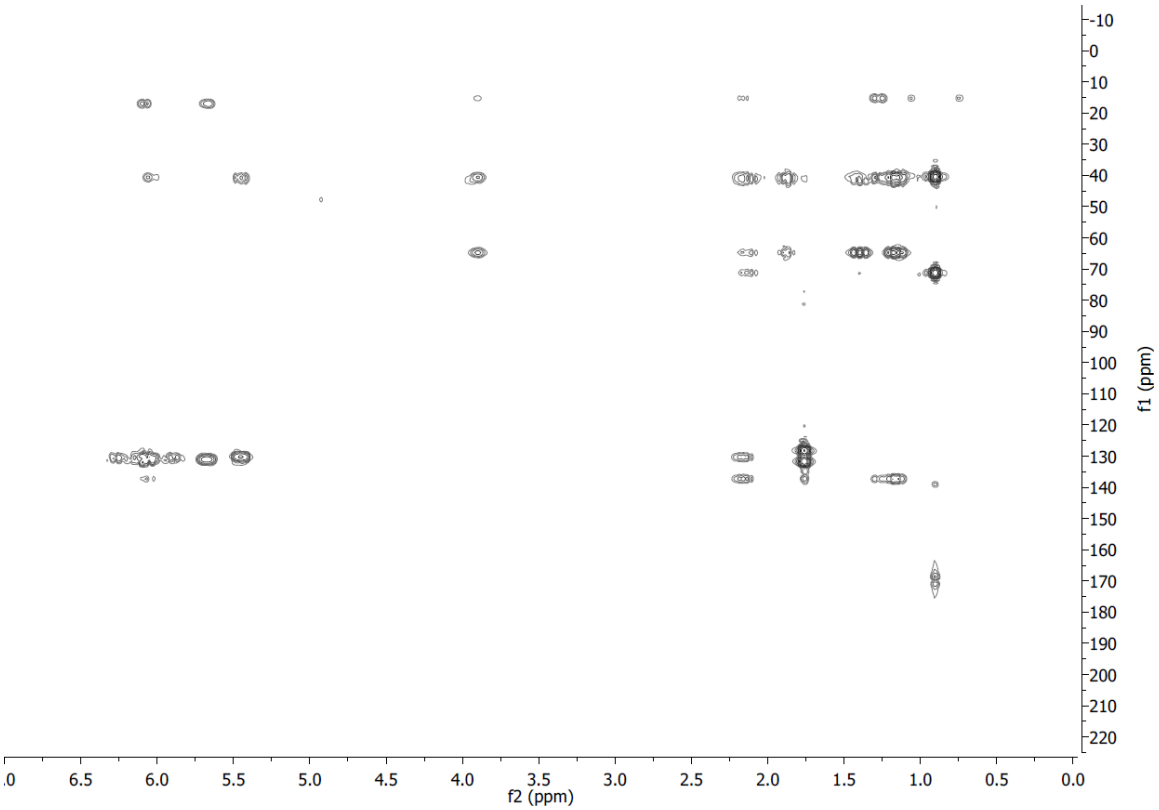


Figure S36. HMBC-NMR spectrum (400 MHz, CD_3OD) waikialide B **(16).**

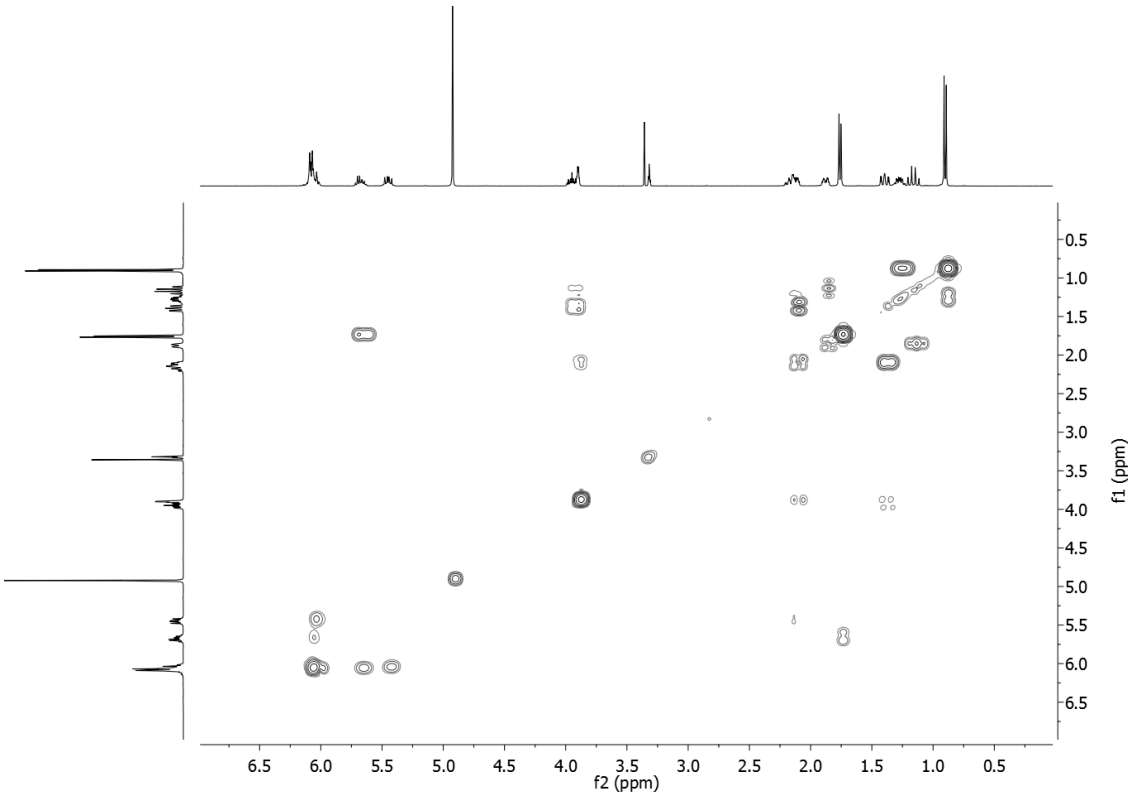


Figure S37. COSY-NMR spectrum (400 MHz, CD_3OD) of waikialide B **(16).**

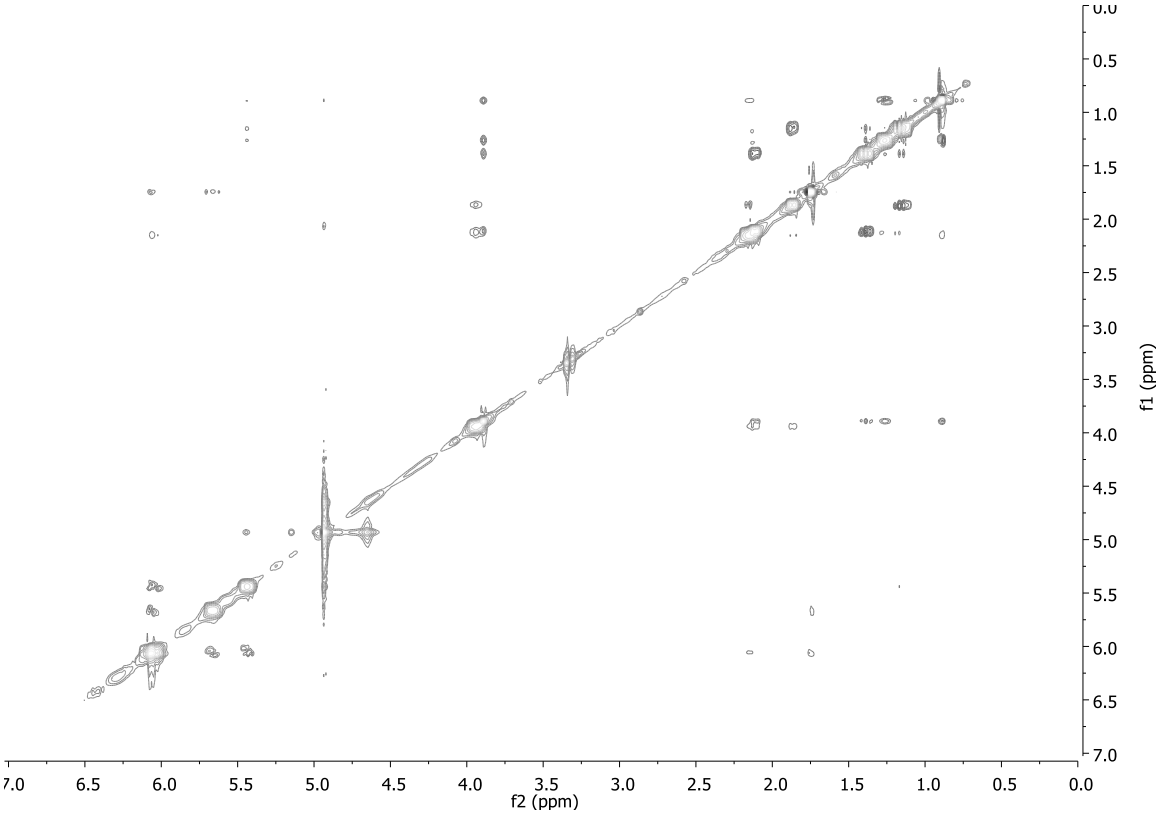


Figure S38. NOESY-NMR spectrum (400 MHz, CD_3OD) of waikialide B **(16).**

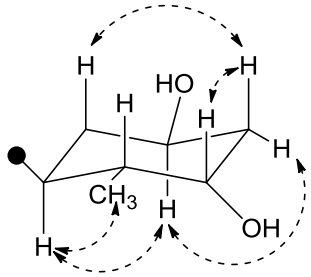


Figure S39. ^{1}H-^{1}H NOESY correlations (dashed double-headed arrows) used to corroborate the relative configuration of the substructure in metabolite **16**.

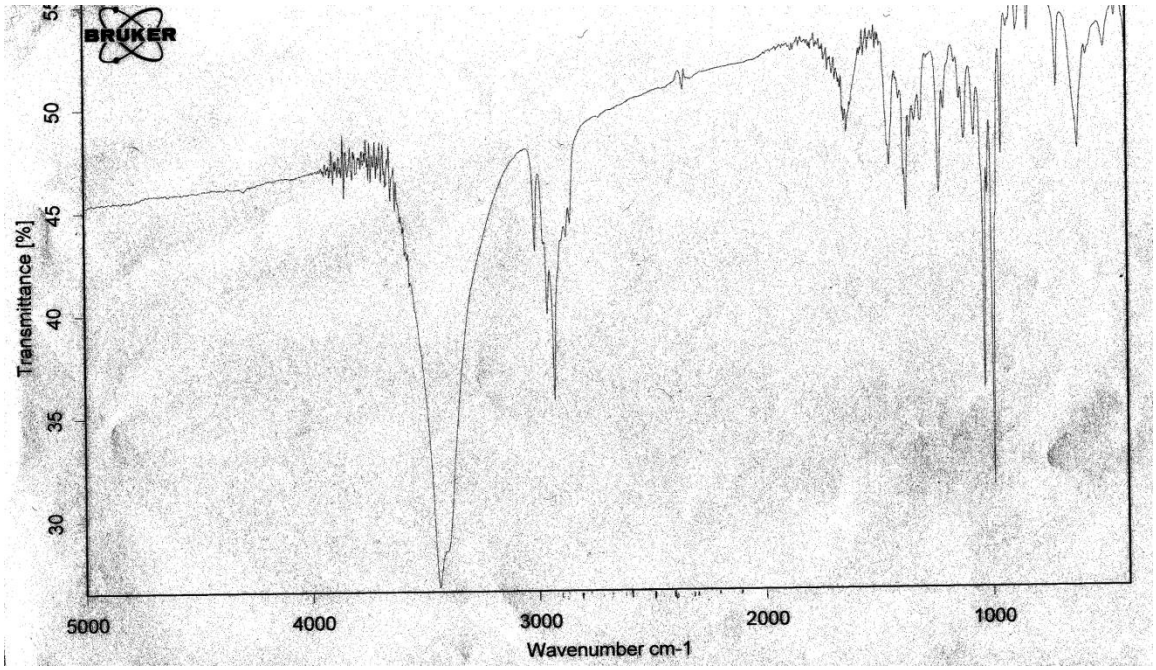


Figure S40. FTIR spectrum of waikialide B **(16).**

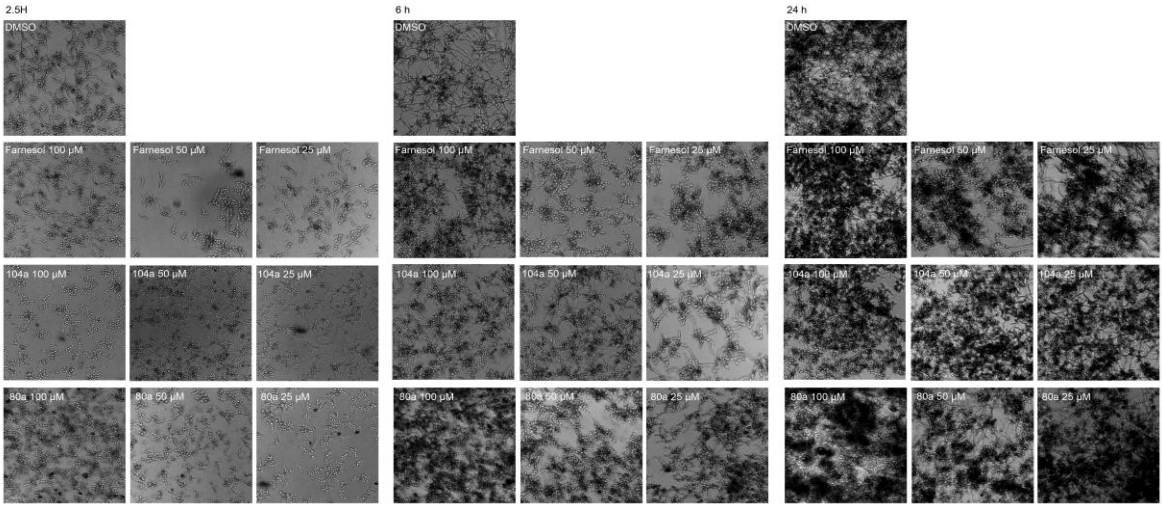


Figure S41. Effects of compounds **1** and **15** on *Candida albicans* hyphae formation. *C.albicans* DAY185 cells were treated with DMSO as negative control, farnesol as positive control, compound **1**, or compound **15**. Cell morphology was visualized at 2.5, 6 and 24 h using a phase contrast microscope (magnification, ×200).

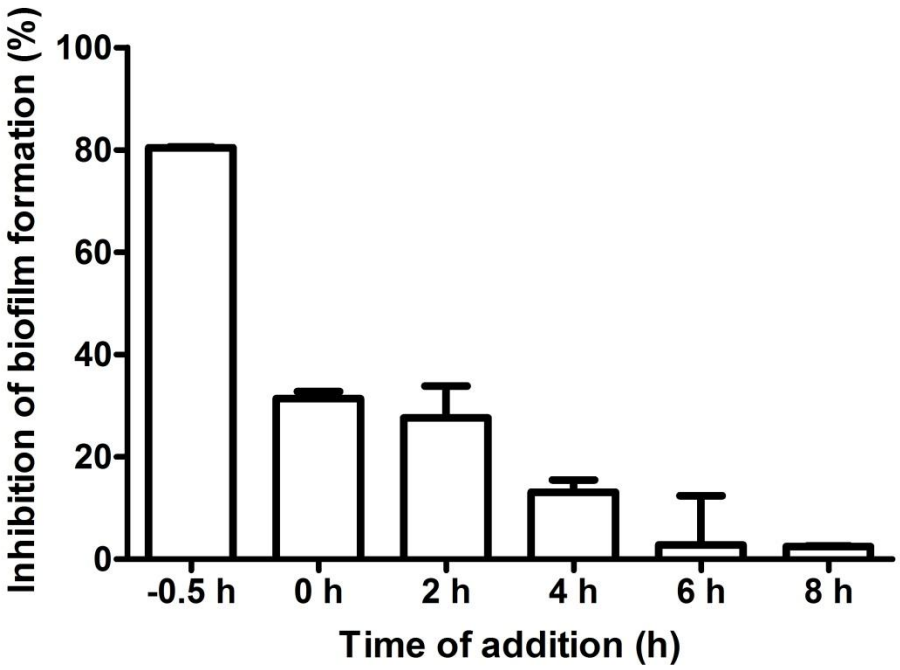


Figure S42. Time of addition assay for compound **1**. Compound **1** (3 μM) was added before (-0.5 h) or after (0, 2, 4, 6, and 8 h) seeding to *C. albicans* DAY185 culture in a 96-well microplate. At 48 h after seeding, the wells were washed twice with PBS and the amount of biofilm formation in the wells was determined using XTT assay. All experiments were performed in triplicate on three separate occasions.

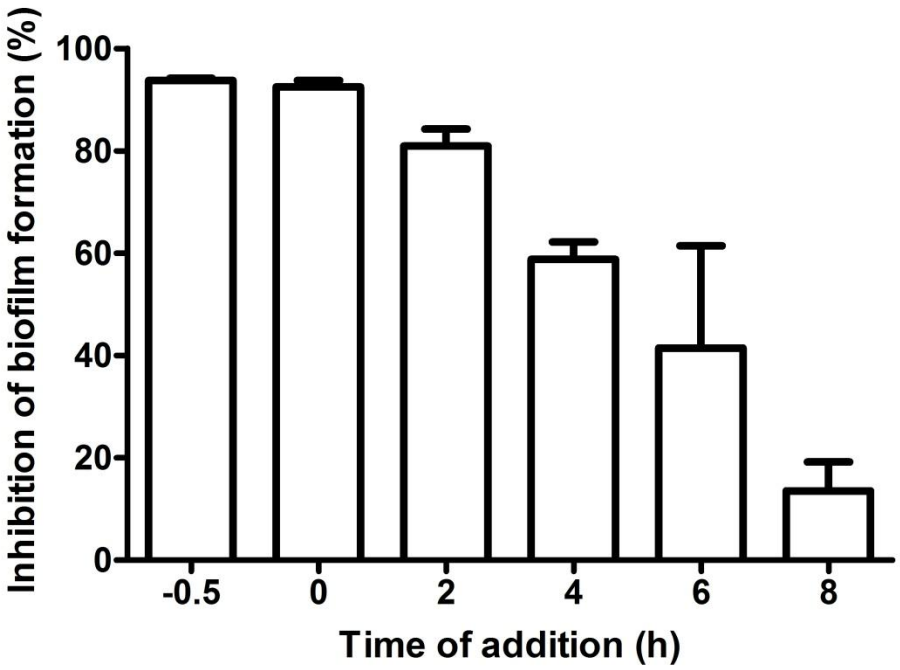


Figure S43. Time of addition assay for compound **1**. Compound **1** (25 μM) was added before (-0.5 h) or after (0, 2, 4, 6, and 8 h) seeding to *C. albicans* DAY185 culture in a 96-well microplate. At 48 h after seeding, the wells were washed twice with PBS and the amount of biofilm formation in the wells was determined using XTT assay. All experiments were performed in triplicate on three separate occasions.