Structural elucidation and antimicrobial characterization of novel diterpenoids from *Fabiana densa* var. *ramulosa*

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1. Chemistry experimental section

1.1 General methods and instrumentation

All non-aqueous reactions were performed under an argon atmosphere using flame-dried glassware and standard syringe/septa techniques.

All absolute solvents were purchased as anhydrous grade from Sigma Aldrich and used without further purification unless otherwise stated. Solvents for extractions, flash column chromatography (FC) and thin layer chromatography (TLC) were purchased as commercial grade from Sigma Aldrich and used without further purification unless otherwise stated. Reactions were magnetically stirred and monitored by TLC performed on Merck TLC aluminum sheets (silica gel 60 F254). Spots were visualized with UV light (λ = 254 nm). Chromatographic purification of products (FC) was performed using Sigma Aldrich silica gel 60 for preparative column chromatography (particle size 40-63 μm).

Melting points (**Mp**) were obtained in open capillary tubes usinga Büchi melting point apparatus B-545 and are uncorrected.

^{1}H NMR and ^{13}CNMR spectra were recorded in $CDCl_3$, acetone-d_6, DMSO-d_6 or methanol-d_4 on a Bruker AV-400 400 MHz spectrometer (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C) at room temperature and tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are

reported in parts per million (ppm) and are referenced to $CDCl_3$ (δ = 7.26 ppm for ^1H, δ = 77.16 ppm for ^{13}C), acetone-d_6 (δ = 2.05 ppm for ^1H, δ = 29.84 ppm for ^{13}C) DMSO-d_6 (δ = 2.50 ppm for ^1H, δ 39.52 ppm for ^{13}C), or MeOH-d_4 (δ = 3.31 ppm for ^1H, δ 49.00 ppm for ^{13}C). All ^{13}C-NMR spectra were measured with complete proton decoupling. Data for NMR spectra are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, *J* = coupling constant in Hz.

High-resolution mass spectra (HRMS) were recorded on Bruker BioApex Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.

Mass spectra (HRMS) were recorded on BRUKER Esquire 3000 PLUS (Esi Ion Trap LC/MSn System)

Plant material Aerial parts of *Fabiana densa* var. *ramulosa* were collected and identified by the School of Chemistry and Pharmacy, University of Chile.

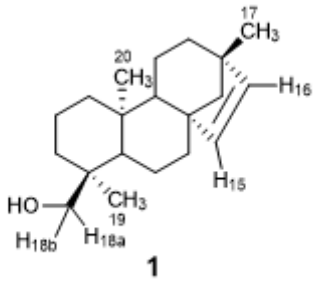
1.2 Procedures and characterization

1.2.1 Extraction and isolation of compounds 1-9

The dried leaves of *Fabiana densa* var. *Ramulosa* (600g) were extracted at room temperature with acetone for 24 h and filtered several times. The filtrate (100g) was subjected to gravity column

chromatography on silica gel and eluted with dichloromethane to give the diterpenic compounds: **5** (0.45%), **6** (0.48%), **7** (0.25%), **1** (*ent-beyer-15-en-18-ol)* (10%) and a fraction (15 g) made by a mixture of five compounds. This fraction was subjected to a flash column chromatography on silica gel with a gradient mobile phase of $CHCl_3$/MeOH obtaining five compounds: **8** (2%), **9** (1%) and **4** (*ent-beyer-15-en-18-O-succinate*) (20%) were obtained by elution with $CHCl_3$/MeOH (98: 2); **3** (*ent-beyer-15-en-18-O-malonate*) (10%) was obtained by elution with $CHCl_3$/MeOH (97: 3); **2** *(ent-beyer-15-en-18-O-oxalate*) (5%) was obtained by elution with $CHCl_3$/MeOH/ formic acid (97: 3: 1).

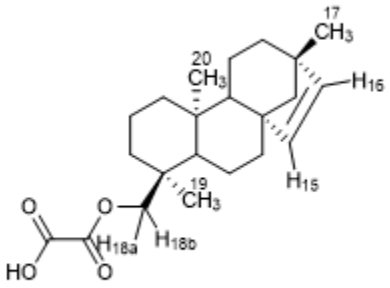
1.2.2 Characterization of compounds 1-9



1

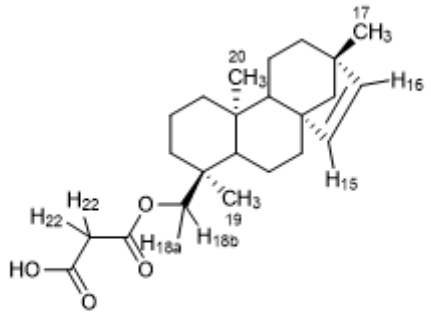
1. ***ent*-beyer-15-en-18-ol.** White powder (yield 10%); m.p. 110 ºC ± 0.5 ºC; $[\alpha]_D$ +29.7° ($CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$,) δ 5.69 (d, 1H, J= 5.7 Hz, H-15), 5.45 (d, 1H, J= 5.6 Hz, H-16), 3.41 (d, 1H, J= 10.8 Hz, H-18a), 3.11 (d, 1H,J= 10.8 Hz, H-18b), 0.99 (s, 3H, CH_3-17), 0.79 (s, 3H, CH_3-19), 0.77 (s, 3H, CH_3-20); **^{13}C NMR** (100 MHz, $CDCl_3$) δ136.55, 135.34, 72.44, 61.31, 52.91, 49.16, 49.11, 43.77, 38.87, 37.70, 37.25, 37.12,

35.50, 33.31, 25.08, 20.35, 19.99, 18.08, 17.92, 15.72; **ESI-MS** (positive) m/z: calcd. for $C_{20}H_{32}ONa$ 311.2, found 311.3 $[M+Na]^+$.



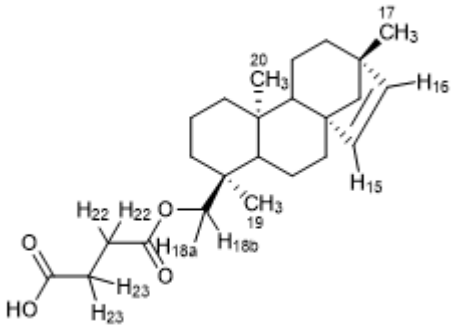
2

2. *ent*-beyer-15-en-18-O-oxalate. White powder (yield 5%); m.p. 169.5 ºC ± 0.5 ºC; $[\alpha]_D$+10° ($CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$,) δ 5.67 (d, 1H, J= 5.7 Hz, H-15,), 5.46 (d, 1H, J= 5.7 Hz, H-16,), 4.10 (d, 1H, J= 10.8 Hz, H-18a), 3.90 (d, 1H, J= 10.8 Hz, H-18b), 0.99 (s, 3H, CH_3-17), 0.91 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-20); **^{13}C NMR** (100 MHz, $CDCl_3$,) δ 158.65, 157.77, 136.74, 135.13, 76.55, 61.17, 52.83, 50.08, 49.01, 43.76, 38.57, 37.36, 36.99, 36.94, 35.92, 33.22, 25.03, 20.35, 20.29, 17.78, 17.62, 15.63; **ESI-HRMS** (negative) m/z: calcd. for $C_{22}H_{31}O_4$ 359.22278, found 359.22245 $[M-H]^-$.



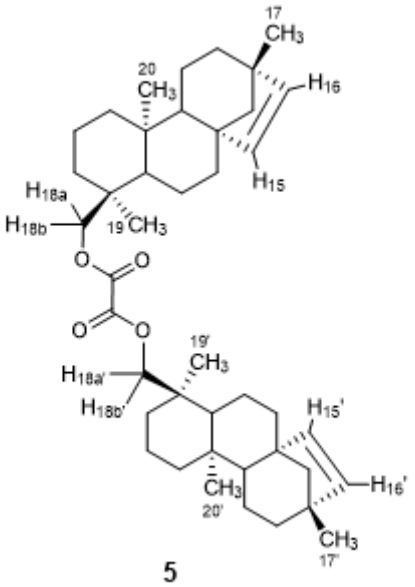
3

3. *ent*-beyer-15-en-18-O-malonate. White oil (yield 10%); $[\alpha]_D$+25.8° ($CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$,) δ 5.67 (d, 1H, J= 5.6 Hz H-15), 5.45 (d, 1H, J= 5.6 Hz, H-16), 3.98 (d, 1H, J= 10.8 Hz, H-18a), 3.75 (d, 1H, J= 10.08 Hz, H-18b), 3.45 (s, 2H, H-22), 0.99 (s, 3H, CH_3-17), 0.85 (s, 3H, CH_3-19), 0.78 (s, 3H, CH_3-20); **^{13}C NMR** (100 MHz, $CDCl_3$,) δ 169.30, 167.81, 136.66, 135.19, 74.66, 61.23, 52.92, 49.89, 43.76, 38.70, 37.32, 36.98, 36.77, 35.95, 33.26, 25.05, 22.83, 20.30, 20.24, 17.86, 17.72, 15.63; **ESI-HRMS** (negative) m/z: calcd. for $C_{23}H_{33}O_4$ 373.23843, found 373.23862 $[M-H]^-$.



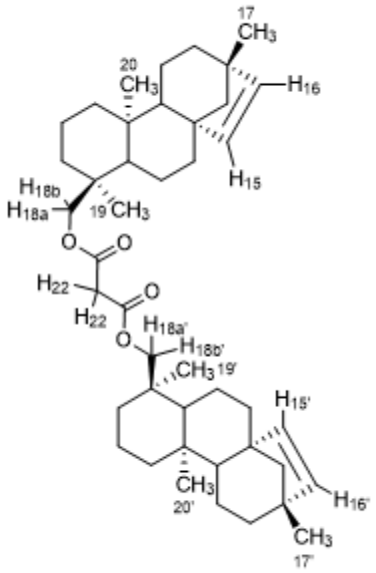
4

4. *ent*-beyer-15-en-18-O-succinate. Brown powder (yield 20%); m.p. 107.5 ºC ± 0.5 ºC; $[\alpha]_D$+14.6° ($CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$,) δ 5.67 (d, 1H, J= 5.6 Hz, H-15), 5.45 (d, 1H, J= 5.6 Hz, H-16), 3.88 (d, 1H, J= 10.8 Hz, H-18a), 3.68 (d, 1H, J= 10.08 Hz, H-18b), 2.67 (m, 4H, H-22,H-23), 0.99 (s, 3H, CH_3-17), 0.83 (s, 3H, CH_3-19), 0.77 (s, 3H, CH_3-20); **^{13}C NMR**(100 MHz, $CDCl_3$,) δ177.44, 172.28, 136.60, 135.25, 73.61, 61.25, 52.93, 50.01, 49.05, 43.75, 38.74, 37.30, 37.06, 36.70, 36.01, 33.27, 29.20, 29.07, 25.06, 20.31, 20.20, 17.92, 17.80, 15.64; **ESI-HRMS** (negative) m/z: calcd. for $C_{24}H_{35}O_4$ 387.25408, found 387.25427 $[M-H]^-$.



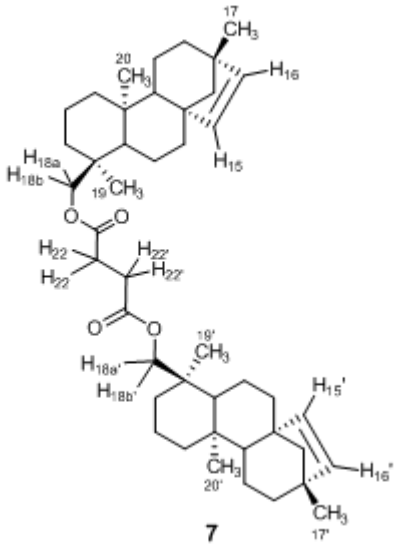
5

5. ***ent*-beyer-15-en-18-O-oxaloyl dimer.** Oil (yield 0.45%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.68 (d, 2H, J= 5.6 Hz, H-16, H-16'), 5.46 (d, 2H, J= 5.6 Hz, H-15, H-15'), 4.12 (d, 2H, J= 10.8 Hz, H-18a, H-18a'), 3.79 (d, 2H, J= 10.8Hz, H-18b, H-18b'), 0.99 (s, 6H, CH_3-17, CH_3-17'), 0.90 (s, 6H, CH_3-19, CH_3-19'), 0.80 (s, 6H, CH_3-20 e CH_3-20'); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 158.08, 136.69, 135.12, 74.95, 61.20, 52.98, 49.66, 49,04, 43.73, 38.82, 37.38, 37.04, 36.89, 35.99, 33.29, 25.04, 20.31, 20.19, 17.93, 17.83, 15.66; **ESI-HRMS** (positive) m/z: calcd. for $C_{42}H_{62}O_4$ Na 653.45403, found 653.45408 $[M+Na]^+$.



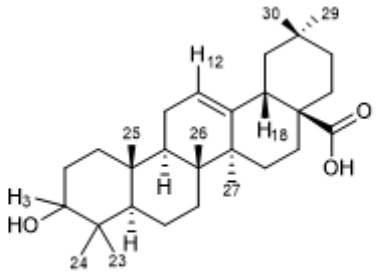
6

6.. *ent*-beyer-15-en-18-O-malonoyl dimer. Oil (yield 0.48%); **^{1}H NMR** (400 MHz, $CDCl_3$,) δ 5.67 (d, 2H, J= 5.6 Hz, H-16, H-16'), 5.45 (d, 2H, J= 5.6 Hz, H-15, H-15'), 3.91 (d, 2H, J= 10.8 Hz, H-18a, H-18a'), 3.78 (d, 2H, J= 10.8 Hz, H-18b, H-18b'), 3.41 (s, 2H, H-22), 0.99 (s, 6H, CH_3-17, CH_3-17'), 0.86 (s, 6H, CH_3-19, CH_3-19'), 0.78 (s, 6H, CH_3-20, CH_3-20'); **^{13}C NMR** (100 MHz, $CDCl_3$,) δ 166.84, 136.64, 135.23, 74.39, 61.28, 52.95, 50.11, 49.05, 43.77, 41.97, 38.72, 37.31, 37.04, 36.72, 36.01, 33.28, 25.06, 20.30, 20.26, 17.89, 17.75, 15.63; **ESI-HRMS** (positive) m/z: calcd for $C_{43}H_{64}O_4Na$ 667.46968, found 667.46911 $[M+Na]^+$.



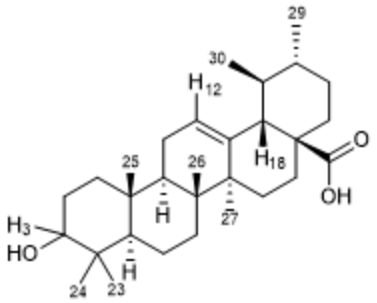
7

7. ***ent*-beyer-15-en-18-O-succinoyl dimer**. White powder (yield 0.25%). m.p. 123 ºC ± 0.5 ºC; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.68 (d, 2H, J= 5.2 Hz, H-16, H-16'), 5.45 (d, 2H, J= 5.2 Hz, H-15, H-15'), 3.88 (d, 2H, J= 10.4 Hz, H-18a, H-18a'), 3.68 (d, 2H, J= 10.4 Hz, H-18b, H-18b'), 2.66 (s, 4H, H-22, H-22'); 0.99 (s, 6H, CH_3-17, CH_3-17'), 0.84 (s, 6H, CH_3-19, CH_3-19'), 0.78 (s, 6H, CH_3-20, CH_3-20'); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 172.45, 136.61, 135.27, 73.61, 61.27, 52.95, 50.11, 49.07, 43.77, 38.78, 37.31, 37.09, 36.70, 36.07, 33.28, 32.08, 29.51, 25.07, 22.85, 20.31, 20.24, 17.94, 17.82, 15.65; **ESI-HRMS** (positive) m/z: calcd.for $C_{44}H_{66}O_4Na$ 681.48533, found 681.48580 $[M+Na]^+$.



8

8. **Oleanolic acid.** White powder (yield 2%); m.p. 309ºC ± 0.5 ºC; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.28 (t, 1H, *J*= 3.4 Hz, H-12), 3.22 (dd, 1H, *J*=10.8, 4.4 Hz, H-3), 2.82 (dd, 1H *J*= 14, 4.4 Hz, H-18), 1.13 (s, 3H, CH_3-27), 0.99 (s, 3H, CH_3-23), 0.93 (s, 3H, CH_3-25), 0.91 (s, 3H, CH_3-30), 0.90 (s, 3H, CH_3-29), 0.77 (s, 3H, CH_3-24), 0.75 (s, 3H, CH_3-26); **^{13}C NMR** (100 MHz, $CDCl_3$,) δ 183.06, 143.73, 122.80, 79.19, 55.38, 47.79, 46.68, 41.77, 41.17, 39.43, 38.91, 37.24, 33.96, 33.21, 32.79, 32.59, 30.82, 28.25, 27.84, 26.08, 23.72, 18.46, 17.25, 15.69, 15.47; **ESI-MS** (negative) m/z: calcd. for $C_{30}H_{47}O_3$ 455, found 455.3 $[M–H]^-$.



9

9. **Ursolic acid.** powder (yield 1%); m.p. 284 ºC ± 0.5 ºC; **^{1}H NMR** (DMSO-d_6, 400 MHz) δ 5.12 (s, 1H,H-12), 3.00 (s, 1H, H-3), 1.04 (s, 3H, CH_3-27), 0.91 (s, 3H, CH_3-30), 0.89 (s, 3H, CH_3-23), 0.86 (s, 3H, CH_3-25), 0.81 (d, 3H, CH_3-29), 0.74 (s, 3H, CH_3-24), 0.67 (s, 3H, CH_3-26); **^{13}C-NMR** (100 MHz, DMSO-d_6,) δ 178.28, 138.19, 124.58, 76.83, 54.78, 52.37, 47.01, 46.82, 41.64, 38.50, 38.44, 38.38, 38.23, 36.53, 36.31, 28.26, 26.99, 23.80, 23.27, 22.85, 21.08, 18.00, 17.01, 16.91, 16.08, 15.23; **ESI-MS** (negative) m/z: calcd. for $C_{30}H_{47}O_3$ 455, found 455.2 $[M - H]^-$.

1.2.3 ^{1}H and ^{13}C NMR spectra of compounds 1-9

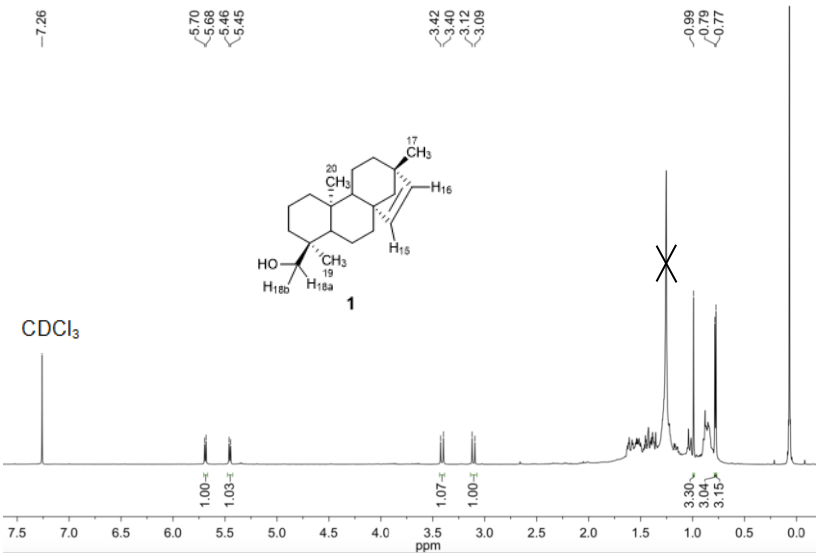


Figure S1. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **1**

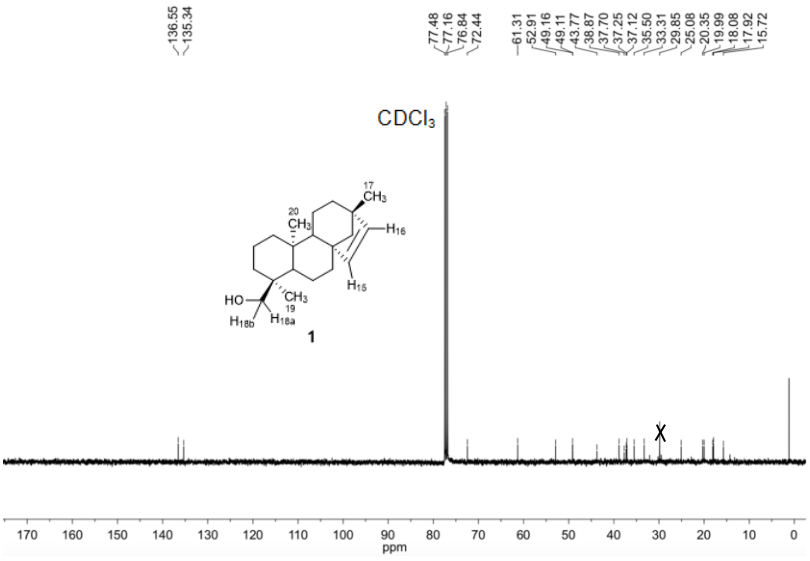


Figure S2. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **1**

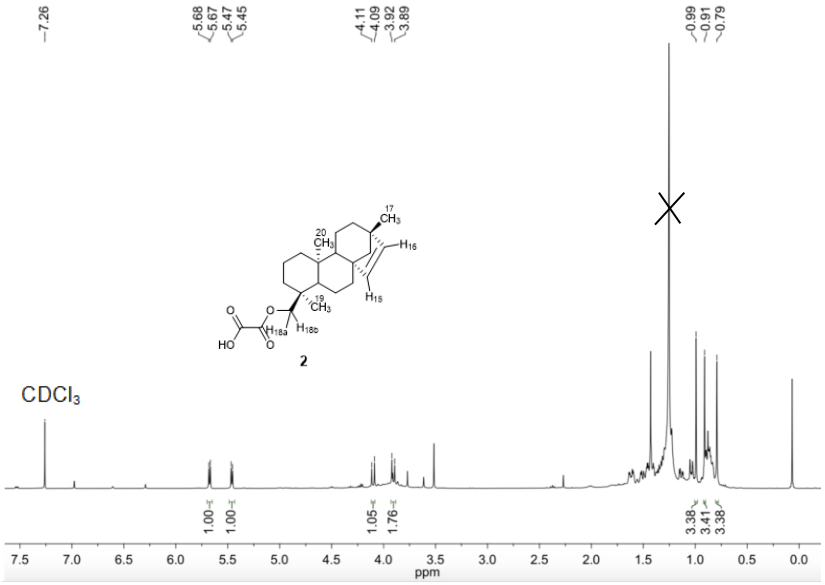


Figure S3. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **2**

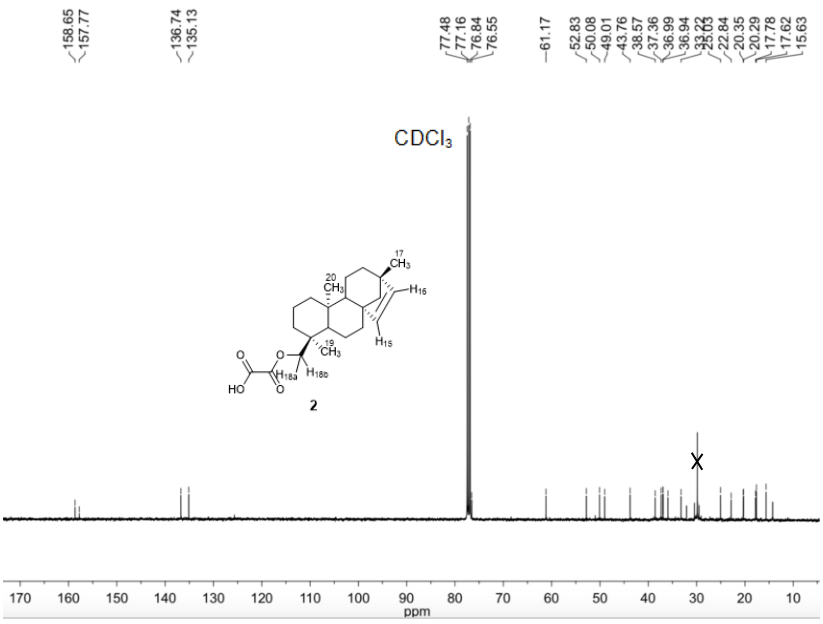


Figure S4. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **2**

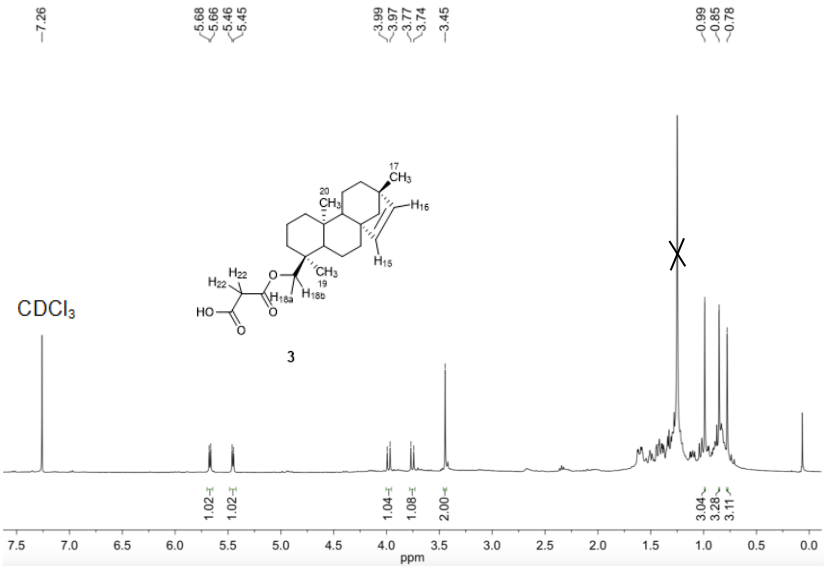


Figure S5. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **3**

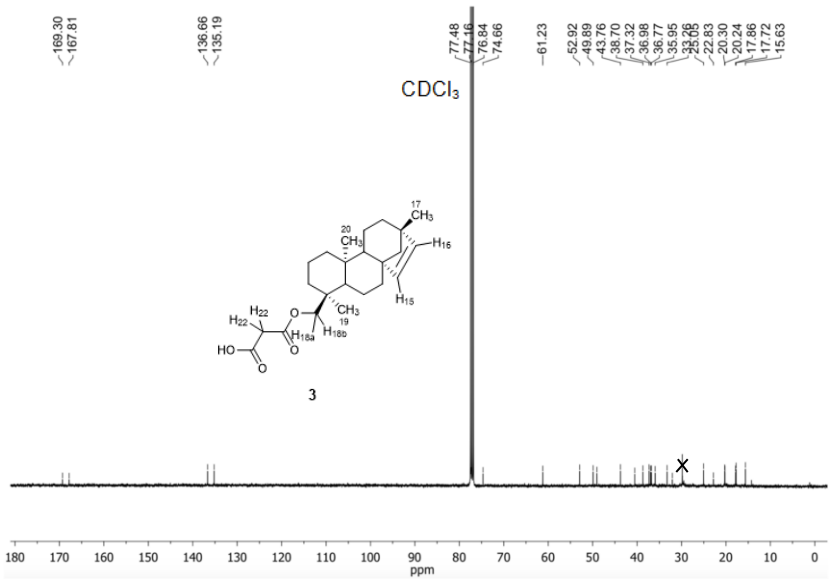


Figure S6. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **3**

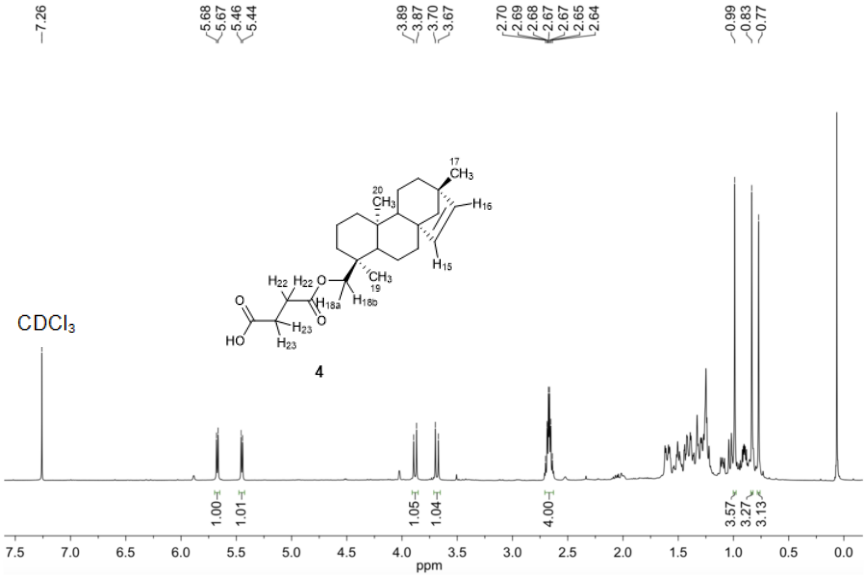


Figure S7. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **4**

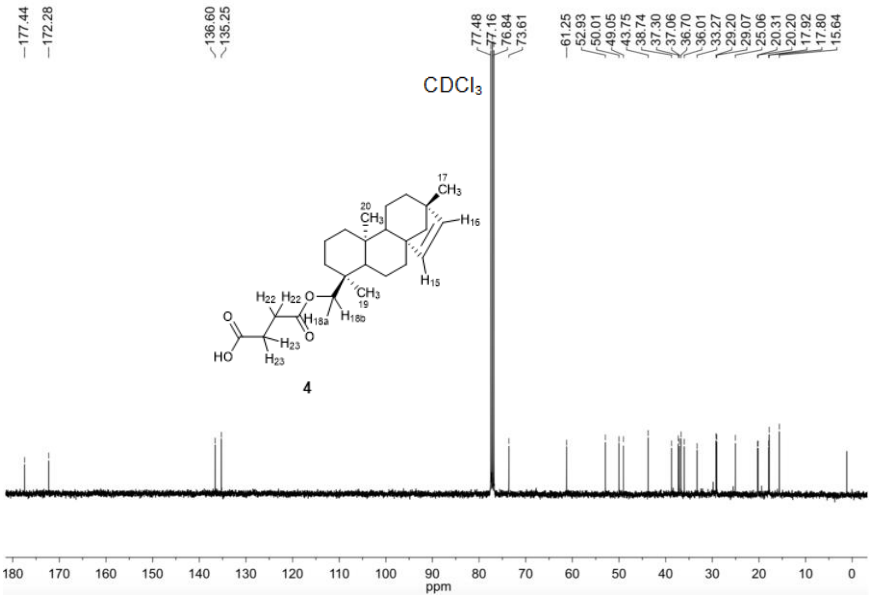


Figure S8. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **4**

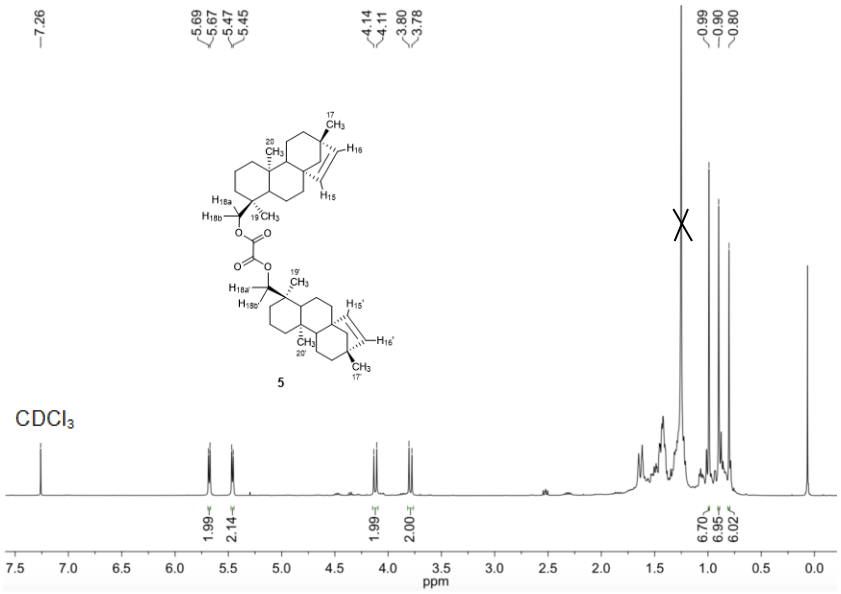


Figure S9. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **5**

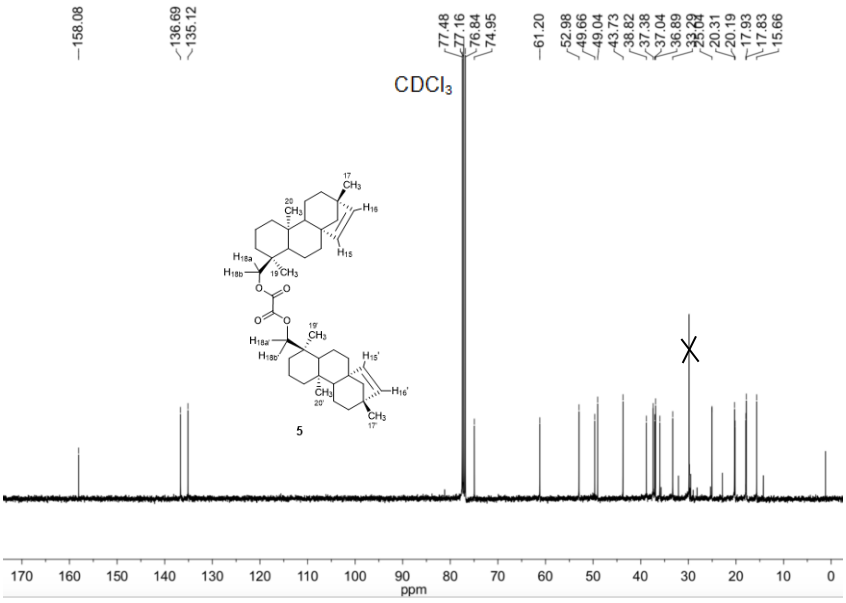


Figure S10. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **5**

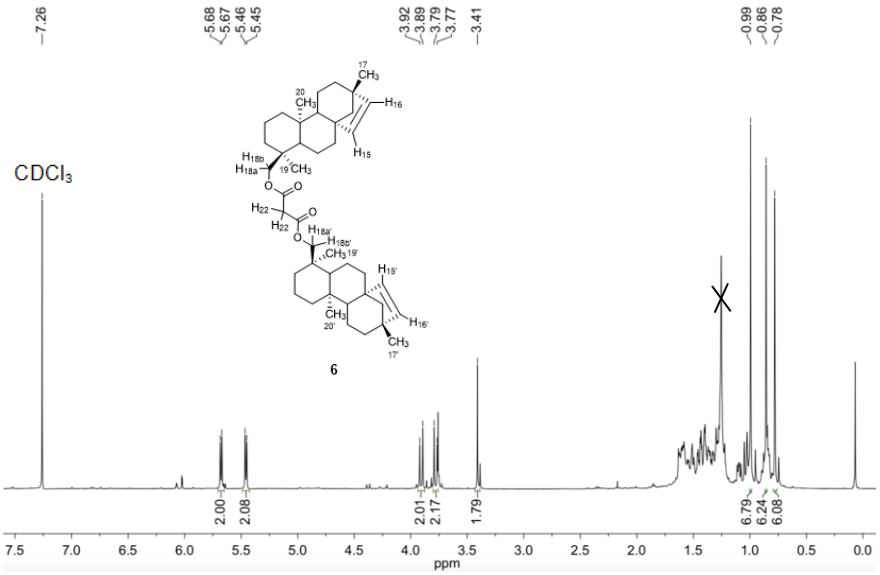


Figure S11. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **6**

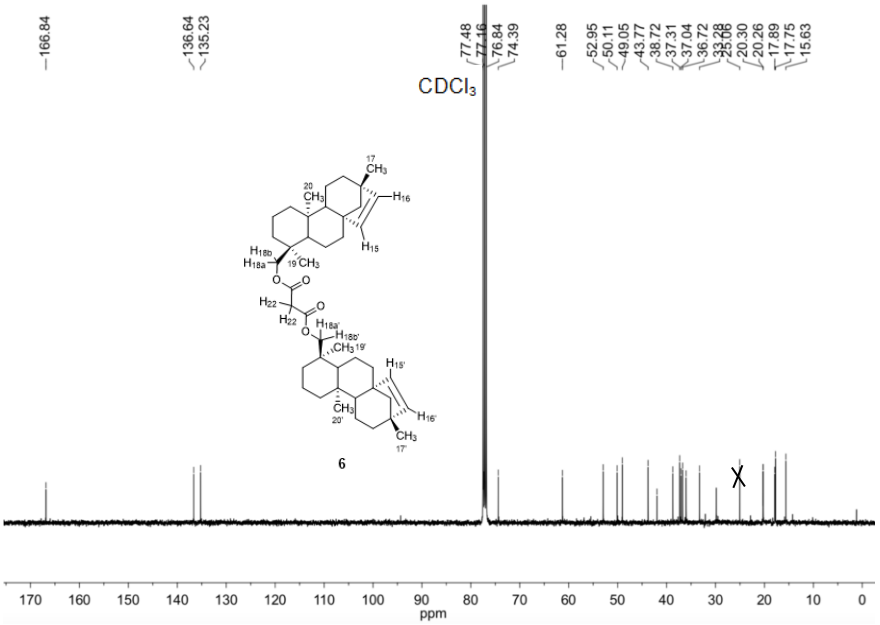


Figure S12. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **6**

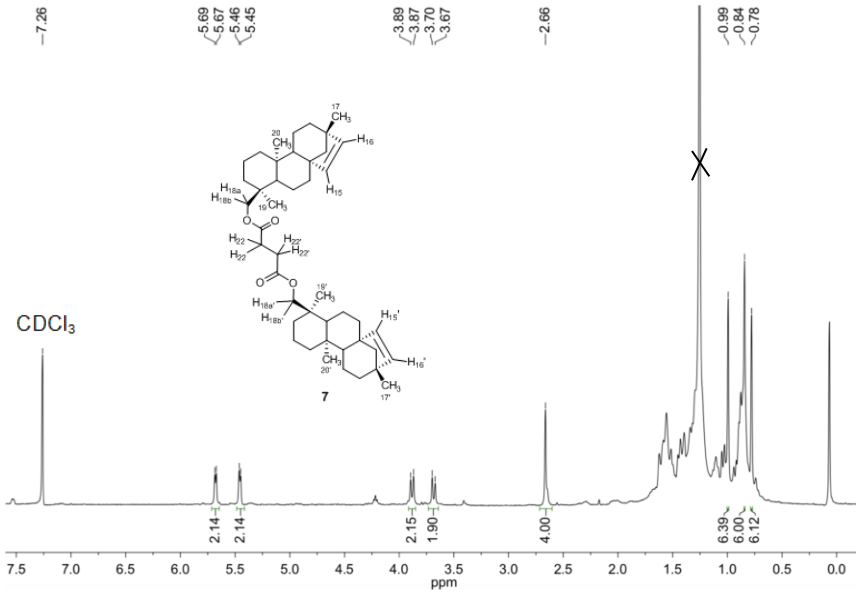


Figure S13. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **7**

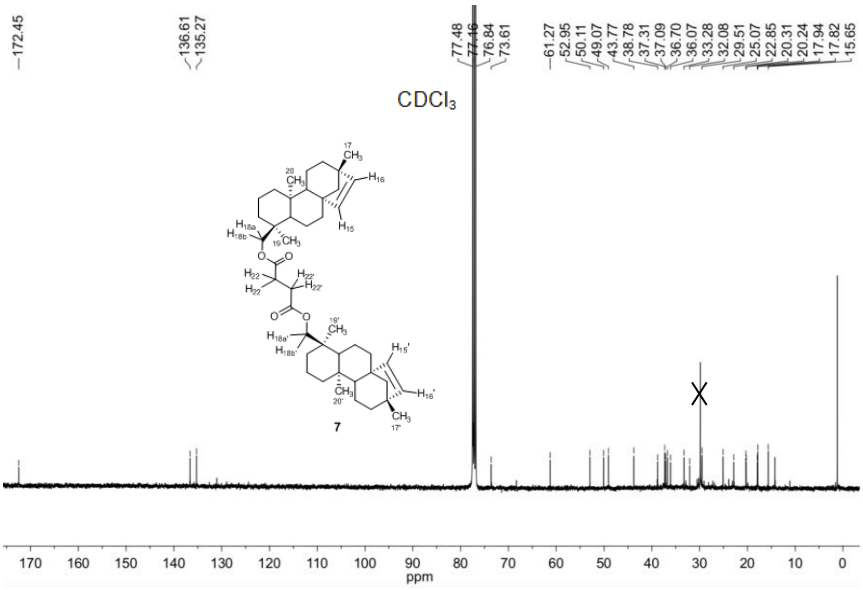


Figure S14. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **7**

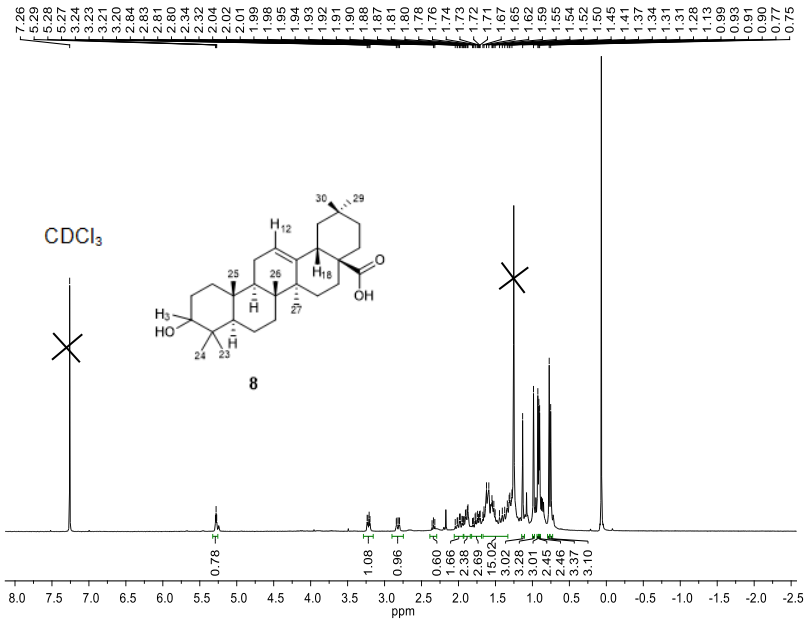


Figure S15. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **8**

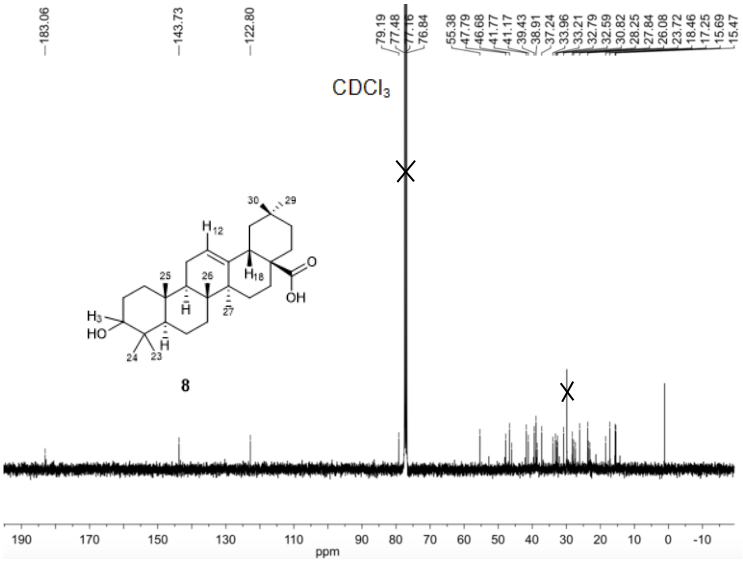


Figure S16. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of compound **8**

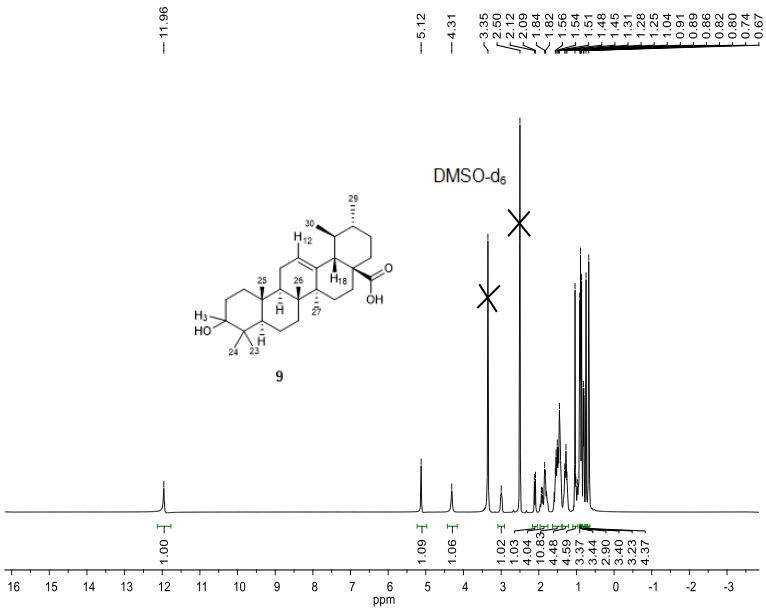


Figure S17. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of compound **9**

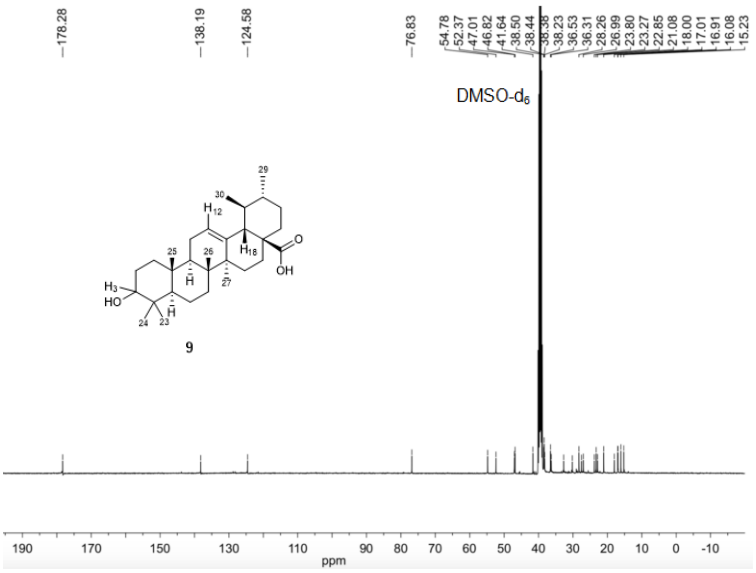


Figure S18. ^{13}C NMR (DMSO-d_6, 100 MHz) spectrum of compound **9**

1.2.4 General procedure to synthesize compounds 2, 3, 4 from 1

To a solution of **1** (0.346 mmol, 99 mg, 1 equiv.) in Et_2O (1.80 ml) the corresponding acyl chloride (0.693 mmol, 4 equiv.) was added dropwise at 0 °C and the reaction mixture was stirred at reflux for 30 minutes. Then the reaction mixture was cooled down and quenched by slow addition of distilled water. The aqueous layer was extracted with Et_2O and the organic layer was washed with water for two times and with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel according to the experimental conditions described previously. The chemical identity of these compounds is assessed by NMR and HRMS experiments which proved to be in agreement with the data reported in paragraph 1.2.2.

2. Biological assays

2.1 Bacterial strains and cell line

The bacterial strains used in this study were the Gram-negative *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922, and the Gram-positive *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *B. megaterium* Bm11 and *B. thuringensis* B15.

The human immortalized keratinocytes cell line, HaCaT, (AddexBio, San Diego, CA, USA) was also used. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4 mM glutamine (DMEMg), 10% heat-inactivated fetal bovine serum (FBS), and 0.1 mg/mL of penicillin and streptomycin, in a humidified atmosphere containing 5% CO_2 at 37 °C, in 25 cm^2 or 75 cm^2 flasks.

2.2 Antimicrobial activity

For each bacterial strain, a fresh inoculum of microbial culture was allowed to grow at 37 °C in Luria-Bertani (LB) medium until it reached an optical density of 0.8 (λ=590 nm). Then, microbial cultures were diluted in Mueller-Hinton (MH) broth at a concentration of 2 x 10^5 colony forming unit (CFU)/mL. Afterwards, aliquots (50 μL) were added to 50 μL of MH where all the selected compounds were properly diluted to reach the desired final concentration ranging from 64 to 16 μM. The plates were incubated at 37 °C with gentle shaking and the microbial growth was evaluated after 16 hours by reading the samples absorbance (at 590 nm) with a microplate reader (Infinite M200; Tecan, Salzburg, Austria).[1] The percentage of microbial growth was calculated with respect to control samples (bacterial cells treated with vehicle, *i.e.* 2-methoxyethanol).

2.3 Cytotoxicity assay

The *in vitro* cytotoxicity of compounds 1, 2, 3 and 4 was evaluated by a colorimetric assay based on the reduction of the yellow dye MTT to its

purple insoluble formazan by mitochondrial dehydrogenases.[2] The intensity of the purple color is directly proportional to the percentage of metabolically-active cells. Briefly, 4 x 10^4 HaCaT cells were resuspended in DMEMg supplemented with 2% FBS and plated in each well of a 96-well microtiter plate. After overnight incubation at 5% CO_2 and 37 °C, the medium was replaced by fresh serum-free DMEMg containing each compound at the desired concentration. For controls, keratinocytes were treated with vehicle (2-methoxyethanol). After incubation for 24 h at 37 °C and 5% CO_2, the medium of each well was replaced by Hank's buffer containing 0.5 mg/mL of MTT. The plate was incubated for additional 4 h; afterwards, the formazan crystals were dissolved by adding acidified isopropanol to each well.[3, 4] The absorbance at 570 nm was measured by a microplate reader (Infinite M200; Tecan, Salzburg, Austria) and the percentage of metabolically-active cells was calculated with respect to the control (cells in medium supplemented with vehicle).

2.4 Statistical analysis

Statistical analysis was performed using Student's t-test and the differences were considered to be statistically significant for $p<0.05$.

3. References

1. Casciaro, B.; Lin, Q.; Afonin, S.; Loffredo, M. R.; de Turris, V.; Middel, V.; Ulrich, A. S.; Di, Y. P.; Mangoni, M. L., Inhibition of Pseudomonas aeruginosa biofilm formation and expression of virulence genes

by selective epimerization in the peptide Esculentin-1a(1-21)NH2. *The FEBS journal* **2019,** *286* (19), 3874-3891.

2. Grieco, P.; Carotenuto, A.; Auriemma, L.; Saviello, M. R.; Campiglia, P.; Gomez-Monterrey, I. M.; Marcellini, L.; Luca, V.; Barra, D.; Novellino, E.; Mangoni, M. L., The effect of d-amino acid substitution on the selectivity of temporin L towards target cells: identification of a potent anti-Candida peptide. *Biochimica et biophysica acta* **2013,** *1828* (2), 652-60.

3. Cappiello, F.; Di Grazia, A.; Segev-Zarko, L. A.; Scali, S.; Ferrera, L.; Galietta, L.; Pini, A.; Shai, Y.; Di, Y. P.; Mangoni, M. L., Esculentin-1a-Derived Peptides Promote Clearance of Pseudomonas aeruginosa Internalized in Bronchial Cells of Cystic Fibrosis Patients and Lung Cell Migration: Biochemical Properties and a Plausible Mode of Action. *Antimicrobial agents and chemotherapy* **2016,** *60* (12), 7252-7262.

4. Di Grazia, A.; Cappiello, F.; Cohen, H.; Casciaro, B.; Luca, V.; Pini, A.; Di, Y. P.; Shai, Y.; Mangoni, M. L., D-Amino acids incorporation in the frog skin-derived peptide esculentin-1a(1-21)NH2 is beneficial for its multiple functions. *Amino acids* **2015,** *47* (12), 2505-19.