Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Lineariifolianoids A–D, rare unsymmetrical sesquiterpenoid dimers comprised by xanthane and guaiane framework units from *Inula lineariifolia*

Jiang-Jiang Qin,[a,c] Ying Huang,[a] Dan Wang,[b] Xiang-Rong Cheng,[a] Qi Zeng,[a] Shou-De Zhang,[a] Zhen-lin Hu,[b] Hui-Zi Jin,*[a] and Wei-Dong Zhang*[a,b]

[a] *School of Pharmacy, Shanghai Jiao Tong University, Dongchuan Rd 800, Shanghai 200240, PR China;*

[b] *School of Pharmacy, Second Military Medical University, Shanghai 200433, PR China.*

[c] *Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA*

* *Corresponding author*

E-mail addresses: wdzhangy@hotmail.com; kimhz@sjtu.edu.cn.

Fax: +86 (0)21 8187 1244; Tel: +86 (0)21 8187 1244

Electronic Supplementary Material (ESI) for RSC Advances

Contents

Fig. S12 HMBC spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

Fig. S13 NOESY spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

Fig. S14 ^1H NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

Fig. S15 ^{13}C NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

Fig. S16 DEPT NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

Fig. S17 HSQC spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

Fig. S18 ^1H–^1H COSY spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

Fig. S19 HMBC spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

Fig. S20 NOESY spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

Fig. S21 ^1H NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

Fig. S22 ^{13}C NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

Fig. S23 DEPT NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

Fig. S24 HSQC spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

Fig. S25 ^1H–^1H COSY spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

Fig. S26 HMBC spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

Fig. S27 NOESY spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

Fig. S28 ^1H NMR spectrum of lineariifolianoid D (**4**) recorded at 400 MHz in $CDCl_3$.

Fig. S29 ^{13}C NMR spectrum of lineariifolianoid D (**4**) recorded at 400 MHz in $CDCl_3$.

Fig. S30 DEPT NMR spectrum of lineariifolianoid D (**4**) recorded at 400 MHz in

$CDCl_3$.

Fig. S31 HSQC spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

Fig. S32 $^1H–^1H$ COSY spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

Fig. S33 HMBC spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

Fig. S34 NOESY spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

Experimental procedures

General procedures: 1D and 2D NMR spectra were taken on a Bruker Avance–400 or Avance–500 spectrometers in $CDCl_3$ or CD_3OD with TMS as internal standard. Optical rotations were obtained with a JASCO P-2000 polarimeter. IR spectra were recorded on a Bruker FTIR Vector 22 spectrometer using KBr pellets. ESIMS spectra were recorded on an Agilent–1100–LC/MSD–Trap XCT spectrometer, whereas HRESIMS were performed using a Waters Q–TOF micro mass spectrometer. Column chromatography (CC) was performed on silica gel (100–200, 200–300 mesh, Yantai, China), and Sephadex LH–20 (GE Healthcare Bio-Sciences AB, Sweden). A preparative column (Shimadzu PRC–ODS EV0233) was used for preparative HPLC (Shimadzu LC–6AD).

Plant material: The aerial parts of *I. lineariifolia* were collected in Changfeng County, Anhui Province, PR China, in July 2007, and identified by Prof. Shou-Jin Liu, Anhui University of Traditional Chinese Medicine. A voucher specimen (No.XX20070701) was deposited at School of Pharmacy, Shanghai Jiao Tong University.

Extraction and isolation: The air-dried aerial parts of *I. lineariifolia* (60.0 kg) were powdered and extracted with 95% ethanol three times each for 24 h at room temperature. The solvent was removed in vacuo to afford a crude EtOH extract, which was suspended in H_2O and then partitioned successively with petroleum ether (PE), CH_2Cl_2, EtOAc, and *n*-BuOH, respectively. 150.0 g of the CH_2Cl_2 extract was subjected to silica gel column eluted with gradient CH_2Cl_2/MeOH (1:0 to 1:1) to give 10 fractions (*Fr.1–Fr.10*) based on TLC analysis. *Fr.2* (33.0 g) was chromatographed on silica gel eluted with a gradient of PE/EtOAc (20:1 to 1:1) to afford five subfractions (*Fr.2-1–Fr.2-5*). Compound **1** (1.6 g) was obtained after CC over Sephadex LH-20 (MeOH) from *Fr.2-4*. *Fr.4* (15.5 g) was subjected to a silica gel CC eluted with gradient PE/EtOAc (5:1 to 1:1) to give eleven subfractions (*Fr.4-1–Fr.4-11*). *Fr.4-9* was subjected to CC over Sephadex LH-20 (MeOH) followed

by preparative HPLC (MeOH/H_2O, 65:35) to yield **2** (5.5 mg). *Fr.4-10* was chromatographed on silica gel eluted with gradient CH_2Cl_2/MeOH (1:0 to 1:1) to give five subfractions (*Fr.4-10a–Fr.4-10e*). Compound **3** (5.0 mg) was obtained after preparative HPLC (MeOH/H_2O, 60:40) from *Fr.4-10d*. *Fr.4-10e* was subjected to preparative HPLC (MeOH/H_2O, 60:40) to give **4** (11.0 mg).

Lineariifolianoid A (1) Colorless monoclinic crystals (MeOH); mp 148–151 °C; $[\alpha]_D^{20}$ +81.7 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3437, 2938, 1751, 1637 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 617 $[M + Na]^+$, HRESIMS (positive) *m/z* 617.2746 $[M + Na]^+$ (calcd for $C_{34}H_{42}O_9Na$, 617.2721).

Lineariifolianoid B (2) Colorless gum; $[\alpha]_D^{20}$ +122.5 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3450, 2935, 1742, 1636 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 591 $[M + Na]^+$, HRESIMS (positive) *m/z* 591.2566 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_9Na$, 591.2565).

Lineariifolianoid C (3) Colorless gum; $[\alpha]_D^{20}$ +198.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3441, 2926, 1744, 1638 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 591 $[M + Na]^+$, HRESIMS (positive) *m/z* 591.2587 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_9Na$, 591.2565).

Lineariifolianoid D (4) Colorless gum; $[\alpha]_D^{20}$ +63.3 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3418, 2936, 1763, 1724, 1634 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 591 $[M + Na]^+$, HRESIMS (positive) *m/z* 591.2576 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_9Na$, 591.2565).

Japonicone A (5) The major sesquiterpenoid dimer isolated from the earial parts of *I. japonica.* Its structure has been elucidated in the reported literature (*Bioorg. Med. Chem. Lett.* **2009**, *19*, 710.).

Crystallographic data of Lineariifolianoid A (1) and Japonicone A (5)

Crystallographic data of lineariifolianoid A (**1**) (copper radiation): $C_{34}H_{42}O_9$, H_2O, M = 612.69, Monoclinic, space group P2 (1), a = 10.9631 (3) Å, α = 90°; b = 21.0687 (5) Å, β = 94.4040 (10)°; c = 14.2884 (4) Å, γ = 90°; V = 3290.56 (15) Å^3, Z = 1, D_{calcd} = 1.237 mg/m^3, crystal size 0.342 × 0.311 × 0.205 mm^3. Cu Kα (λ = 1.54178 Å), F (000) = 1312, T = 296(2) K. The final R values were R_1 = 0.0433, and wR_2 = 0.1285, for 11088 observed reflections [I > 2σ (I)]. The absolute structure parameter was 0.04(14).

Crystallographic data of japonicone A (**5**) (copper radiation): $C_{32}H_{40}O_7$, CH_3CN, M = 577.69, Monoclicic, space group P2 (1), a = 9.50250 (10) Å, α = 90°; b = 9.95250 (10) Å, β = 104.11°; c = 17.3337 (2) Å, γ = 90°; V = 1589.82 (3) Å^3, Z = 2, D_{calcd} = 1.207 mg/m^3, crystal size 0.321 × 0.232 × 0.176 mm^3. Cu Kα (λ = 1.54178 Å), F (000) = 620, T = 296(2) K. The final R values were R_1 = 0.0313, and wR_2 = 0.0948, for 12568 observed reflections [I > 2σ (I)]. The absolute structure parameter was 0.10 (14).

Crystallographic data for **1** and **5** have been deposited at the Cambridge Crystallographic Data Centre (deposition number: CCDC 806236 and 823557, respectively). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk).

TNF-α-mediated L929 cytotoxicity assay

L929 cells were seeded in 96-well plates at 2.5×10^4 cells/well and cultures overnight. TNF-α (10 ng/ml) was incubated with different concentrations of lineariifolianoids A–D (**1**–**4**) in culture medium at 37 °C for 30 min. After incubation, the mixtures of TNF-α with lineariifolianoids were added to the cells with 1μg/ml of Actinomycin D and cultured for 16 h. Cell viability was assessed by microscope examination and the MTT colorimetric assay. Percentage inhibition of cytotoxicity was calculated with the following formula: $(OD_{actinomycinD+TNF\text{-}\alpha+Comps} - OD_{actinomycinD+TNF\text{-}\alpha})/(OD_{actinomycinD} - OD_{actinomycinD+TNF\text{-}\alpha}) \times 100$. Each concentration of lineariifolianoids was tested in triplicate.

Table S1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **1–4** in $CDCl_3$

No.	1		2		3		4	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	152.4 s		143.5 s		151.7 s		150.6 s	
2a	31.1 t	2.45 m	30.5 t	2.35 m	30.9 t	2.40 m	31.4 t	2.46 m
2b		2.29 m		2.25 m		2.40 m		2.30 m
3a	42.1 t	2.57 m	42.0 t	2.55 m	42.4 t	2.52 m	42.0 t	2.62 m
3b		2.44 m		2.45 m		2.40 m		2.46 m
4	206.9 s		207.4 s		n.o.		209.6 s	
5	121.3 d	5.73 d (8.3)	132.4 d	5.36 s	121.6 d	5.71 d (8.0)	122.5 d	5.64 d (8.0)
6	68.8 d	5.29 d (8.4)	66.6 d	4.65 d (9.6)	69.1 d	5.38 d (8.7)	70.4 d	4.68 m
7	49.0 d	2.48 m	52.3 d	2.45 m	48.3 d	2.95 m	49.7 d	3.51 d (7.5)
8	78.0 d	4.80 m	78.7 d	4.80 m	79.1 d	4.86 ddd (11.3,5.9,5.9)	81.1 d	4.95 m
9a	38.1 t	2.40 m	36.1 t	2.25 m	38.1 t	2.40 m	37.1 t	2.46 m
9b		2.22 m		1.90 m		2.20 m		2.20 m
10	31.9 d	2.37 m	32.0 d	2.35 m	31.8 d	2.40 m	31.9 d	2.40 m
11	54.4 s		55.8 s		54.8 s		57.6 s	
12	178.0 s		180.1 s		180.7 s		185.6 s	
13a	36.0 t	1.80 m	33.3 t	3.57 d (12.4)	32.7 t	2.40 m	35.3 t	2.46 m
13b		1.80 m		1.66 d (12.8)		1.50 d (12.1)		1.56 d (13.6)
14	21.5 q	1.18 d (6.6)	21.1 q	1.16 d (7.0)	21.4 q	1.18 d (6.7)	21.4 q	1.13 d (6.8)
15	29.9 q	2.13 s	29.9 q	2.14 s	29.8 q	2.14 ovl	29.8 q	2.11 s
1′	62.4 s		60.0 s		60.7 s		72.3 s	
2′	82.1 d	4.57 br s	80.8 d	4.57 s	82.6 d	3.76 s	85.6 d	3.62 d (12.2)
3′	57.0 d	2.81 m	59.1 d	2.85 s	60.3 d	2.60 s	52.7 d	2.58 s
4′	133.8 s		137.4 s		138.4 s		148.2 s	
5′	136.6 s		139.7 s		139.9 s		139.1 s	
6′*α*	26.0 t	3.02 br d (15.9)	69.7 d	4.73 d (6.8)	69.9 d	4.71 d (7.0)	68.4 d	4.69 m
6′*β*		2.05 m						
7′	45.3 d	2.78 m	47.9 d	3.22 m	48.1 d	3.21 m	47.9 d	3.18 m
8′	82.4 d	4.18 m	76.3 d	4.15 dd (17.6, 9.7)	76.4 d	4.12 dd (17.6, 9.7)	75.6 d	4.07 dd (17.8, 9.7)
9′*α*	36.0 t	2.31 m	38.2 t	2.20 m	38.5 t	2.20 m	40.5 t	2.46 m
9′*β*		1.90 m		1.90 m		1.90 m		1.88 m
10′	29.7 d	2.05 m	29.4 d	2.57 m	29.7 d	2.58 m	30.0 d	2.75 m
11′	139.4 s		139.5 s		139.7 s		140.0 s	
12′	170.1 s		169.9 s		170.0 s		169.6 s	
13′a	119.5 t	6.25 d (3.2)	121.0 t	6.26 d (3.0)	121.7 t	6.26 d (3.4)	120.7 t	6.25 d (3.4)
13′b		5.52 d (3.0)		5.95 br s		5.98 br s		5.88 d (2.9)
14′	17.0 q	1.03 d (7.2)	16.6 q	1.07 d (6.9)	17.4 q	1.18 d (6.7)	17.3 q	1.31 d (6.7)
15′	14.4 q	1.66 s	13.7 q	1.71 s	14.1 q	1.69 s	12.9 q	1.81 s
6-OAc	169.9 s				170.1 s		170.2 s	
2′-OH								5.51 d (12.6)
2′-OAc	170.0 s		170.0					

Ovl: overlapped; n.o.: not observed.

Fig. S1 Key NOESY correlations of lineariifolianoid A (**1**).

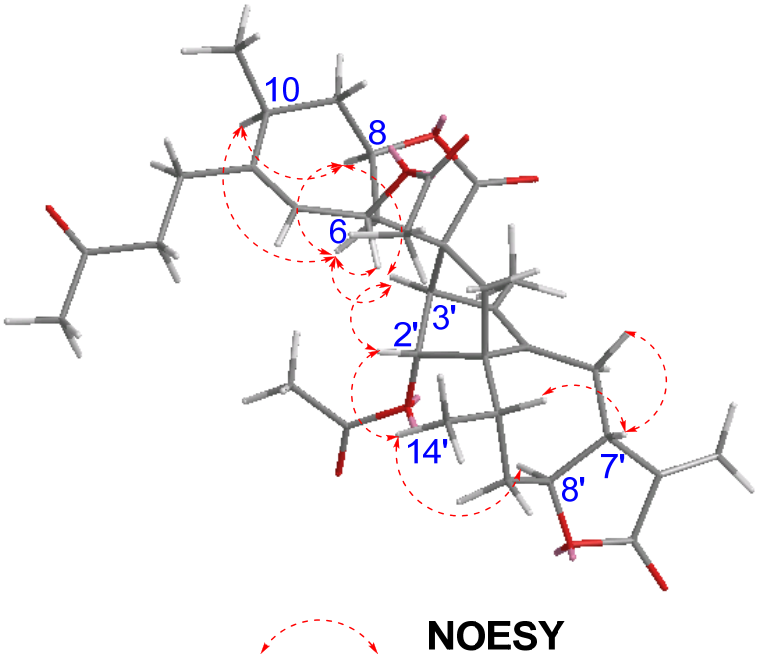

Fig. S2 Key NOESY correlations of lineariifolianoid B (**2**).

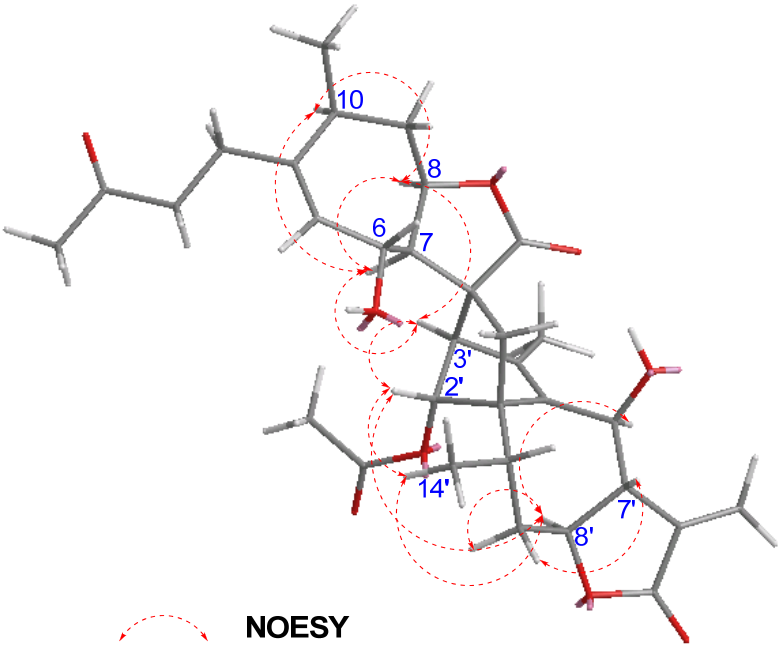

Fig. S3 Key ^{1}H–^{1}H COSY and HMBC correlations of lineariifolianoid B (**2**).

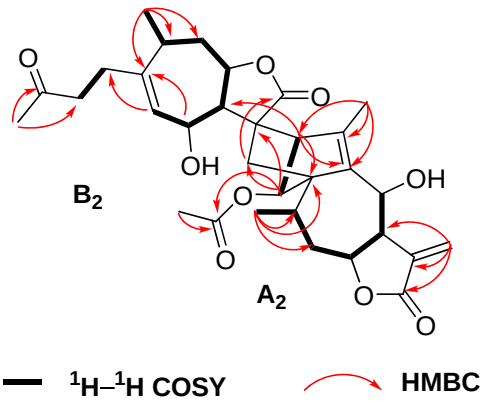

Fig. S4 Key ^{1}H–^{1}H COSY and HMBC correlations of lineariifolianoid C (**3**).

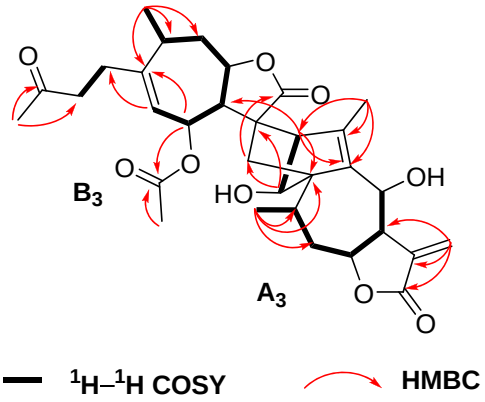

Fig. S5 Key ^{1}H–^{1}H COSY and HMBC correlations of lineariifolianoid D (**4**).

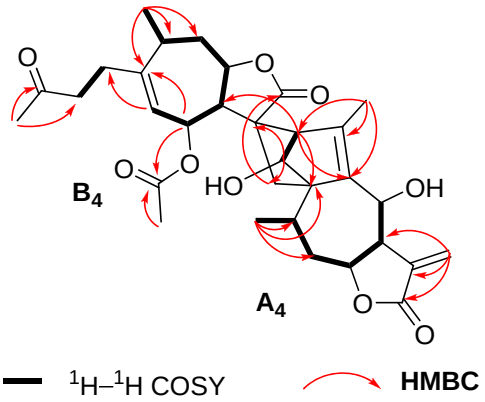

Fig. S6 Different concentrations of lineariifolianoids A–D (**1**–**4**) were premixed with a fixed concentration of TNF-α (10 ng/ml) and were added to L929 cells together with 1 μg/ml of Actinomycin D. After 16 h of incubation, Cell viability was examined under microscope and the inhibition of cytotoxicity was measured by MTT method. The results of MTT assay showing that lineariifolianoid D (**4**) inhibited TNF-α-mediated cytotoxicity dose-dependently from 2.5 to 10 μM.

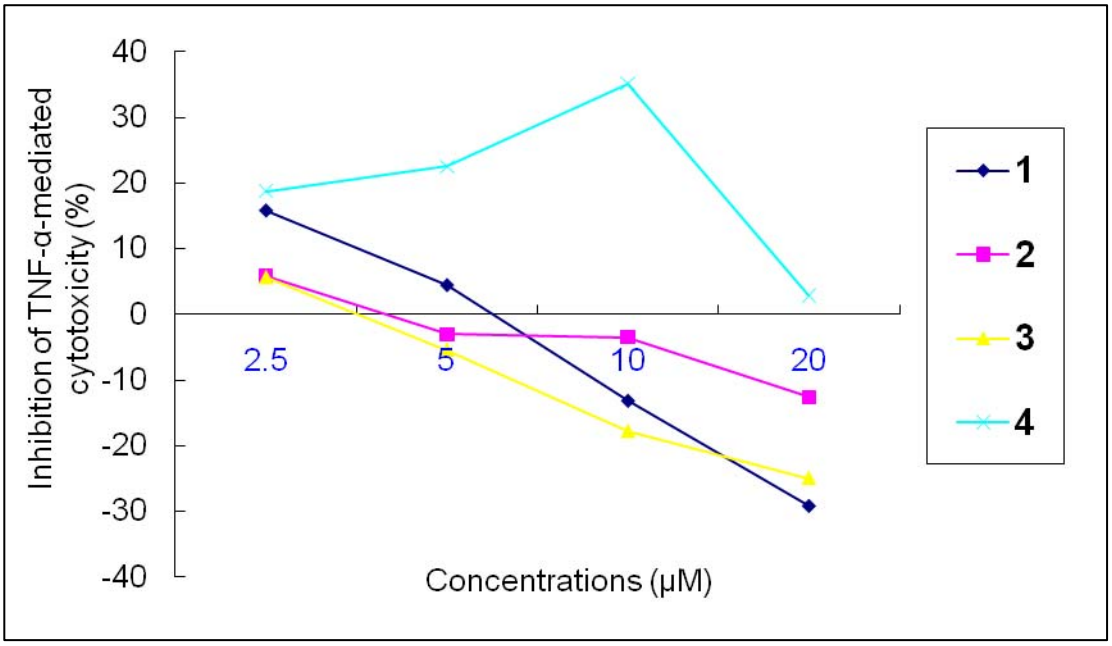

Fig. S7 ^{1}H NMR spectrum of lineariifolianoid A (**1**) recorded at 400 MHz in $CDCl_3$.

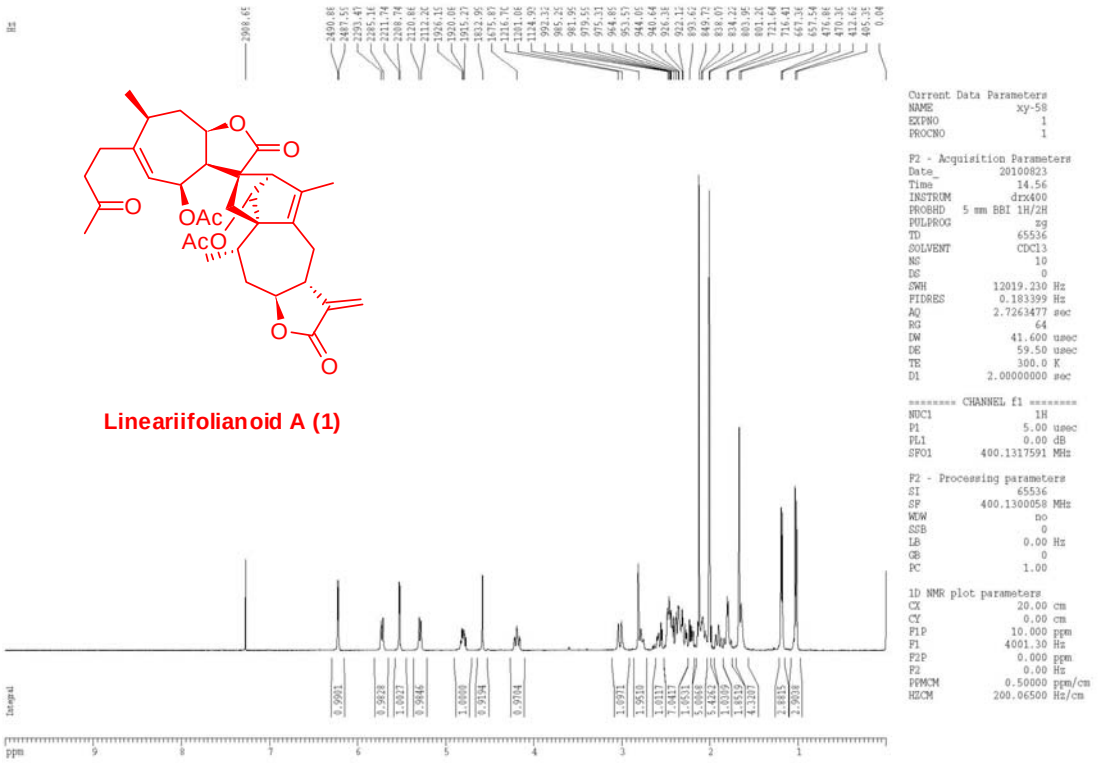

Fig. S8 ^{13}C NMR spectrum of lineariifolianoid A (**1**) recorded at 100 MHz in $CDCl_3$.

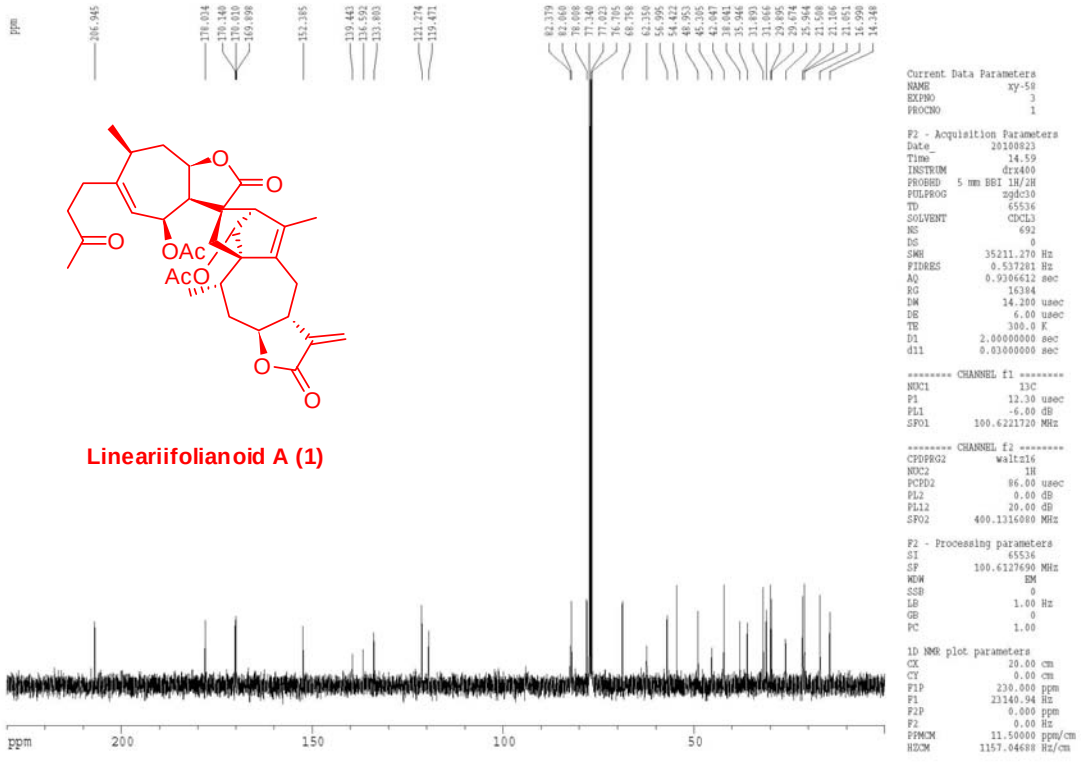

Fig. S9 DEPT NMR spectrum of lineariifolianoid A (**1**) recorded at 100 MHz in $CDCl_3$.

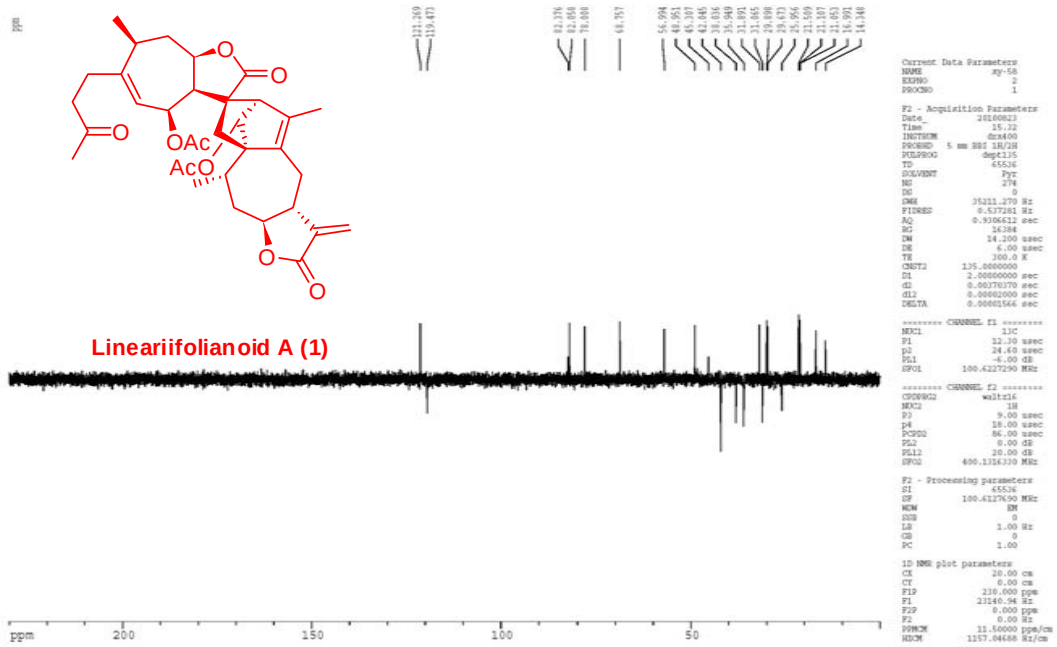

Fig. S10 HSQC spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

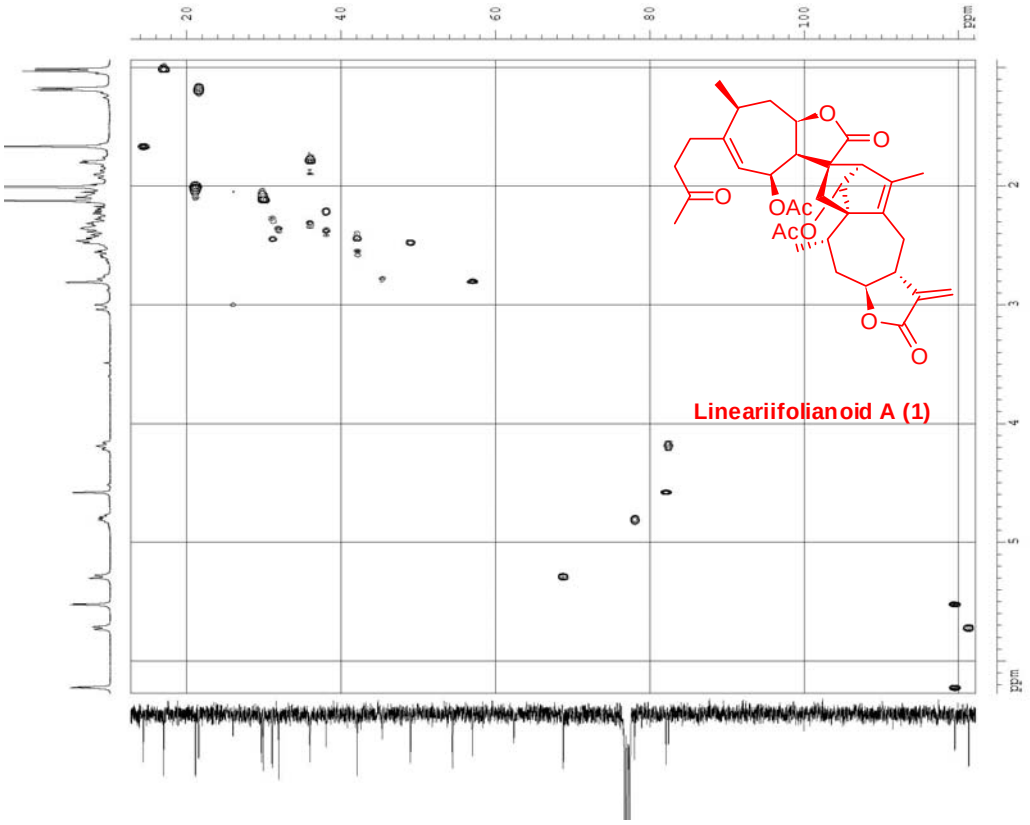

Fig. S11 $^{1}H–^{1}H$ COSY spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

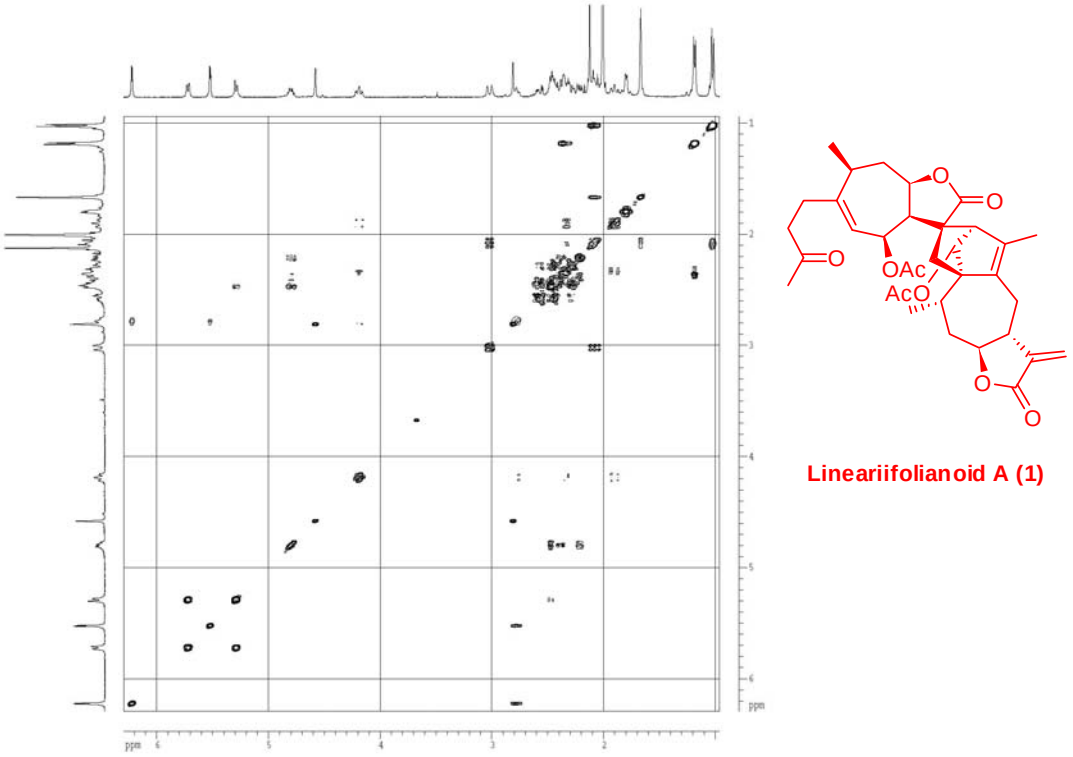

Fig. S12 HMBC spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

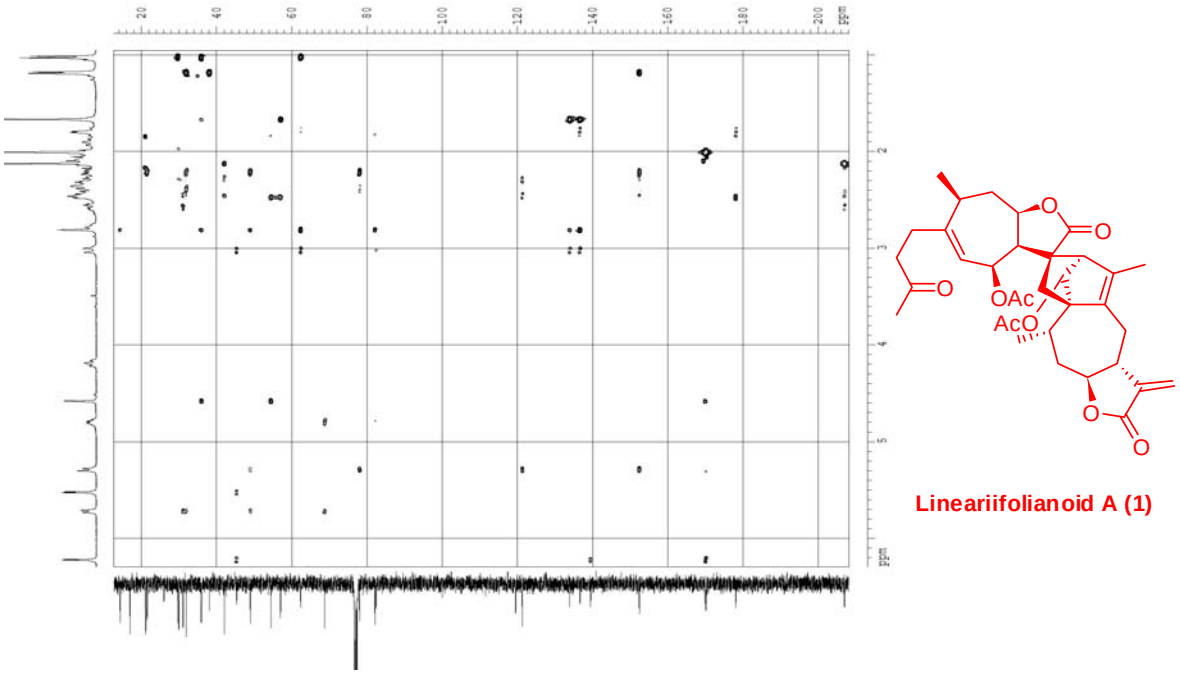

Fig. S13 NOESY spectrum of lineariifolianoid A (**1**) recorded in $CDCl_3$.

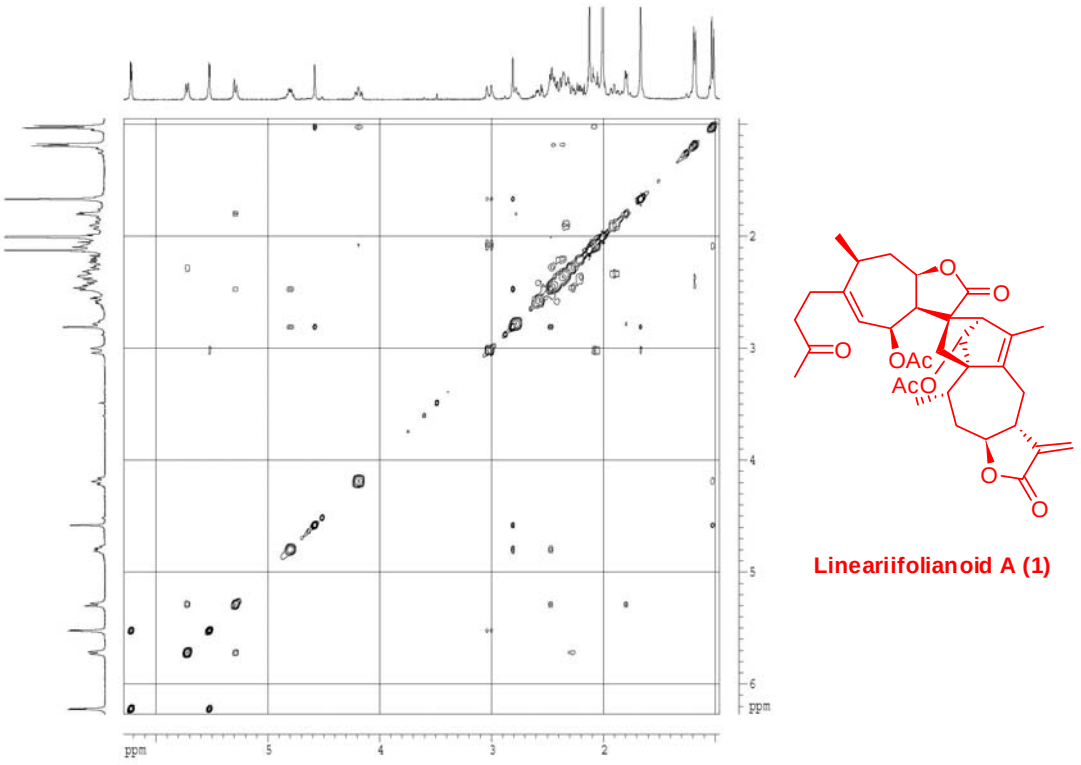

Fig. S14 ^1H NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

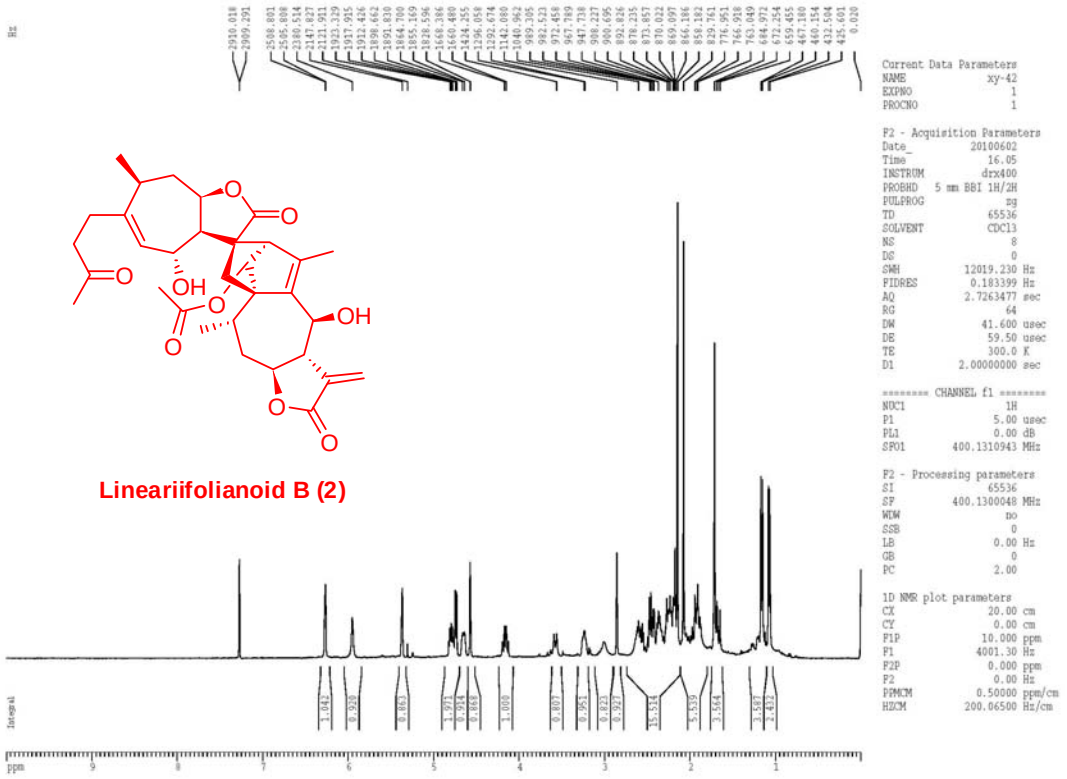

Fig. S15 ^{13}C NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

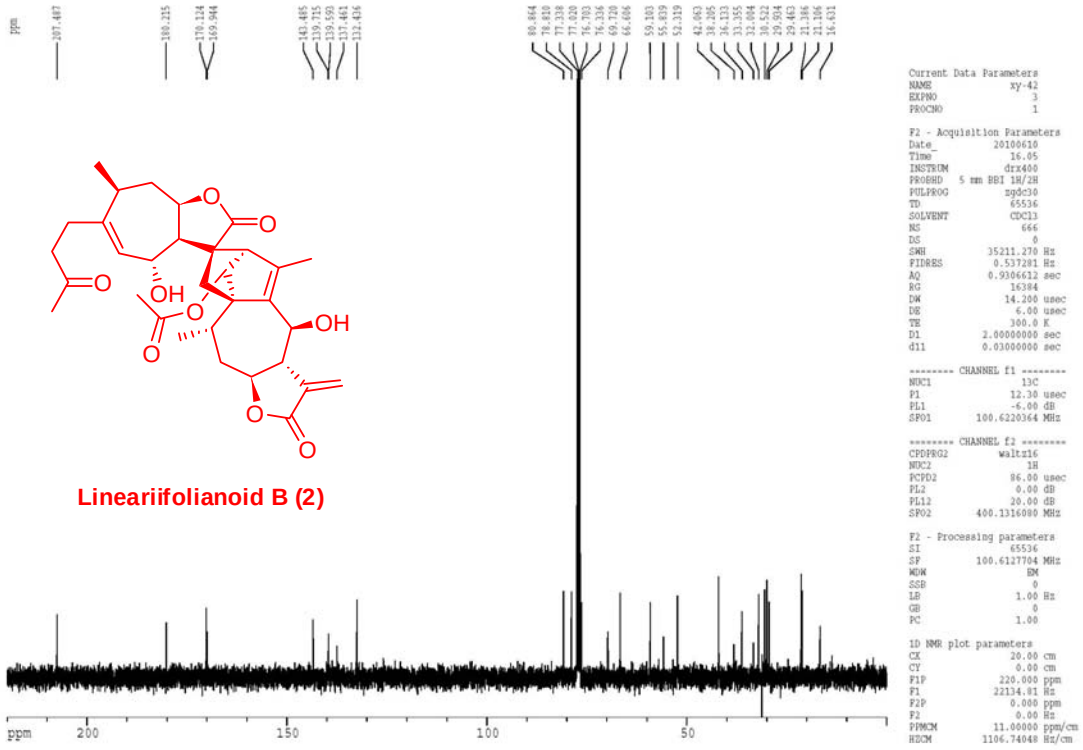

Fig. S16 DEPT NMR spectrum of lineariifolianoid B (**2**) recorded at 400 MHz in $CDCl_3$.

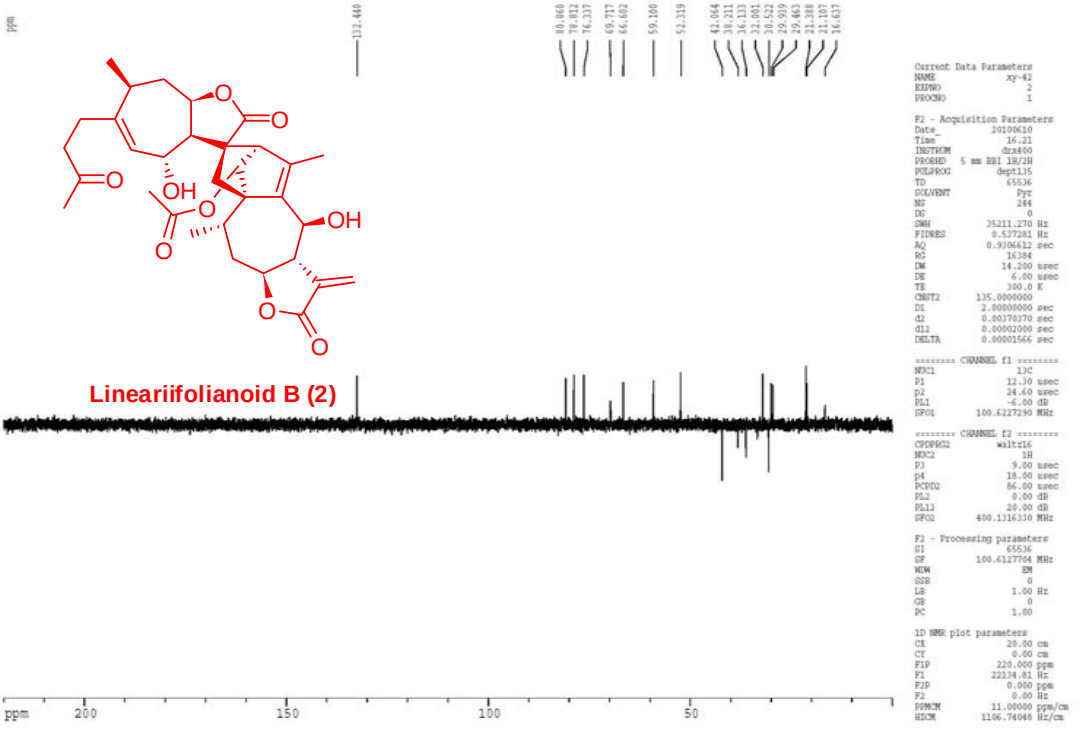

Fig. S17 HSQC spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

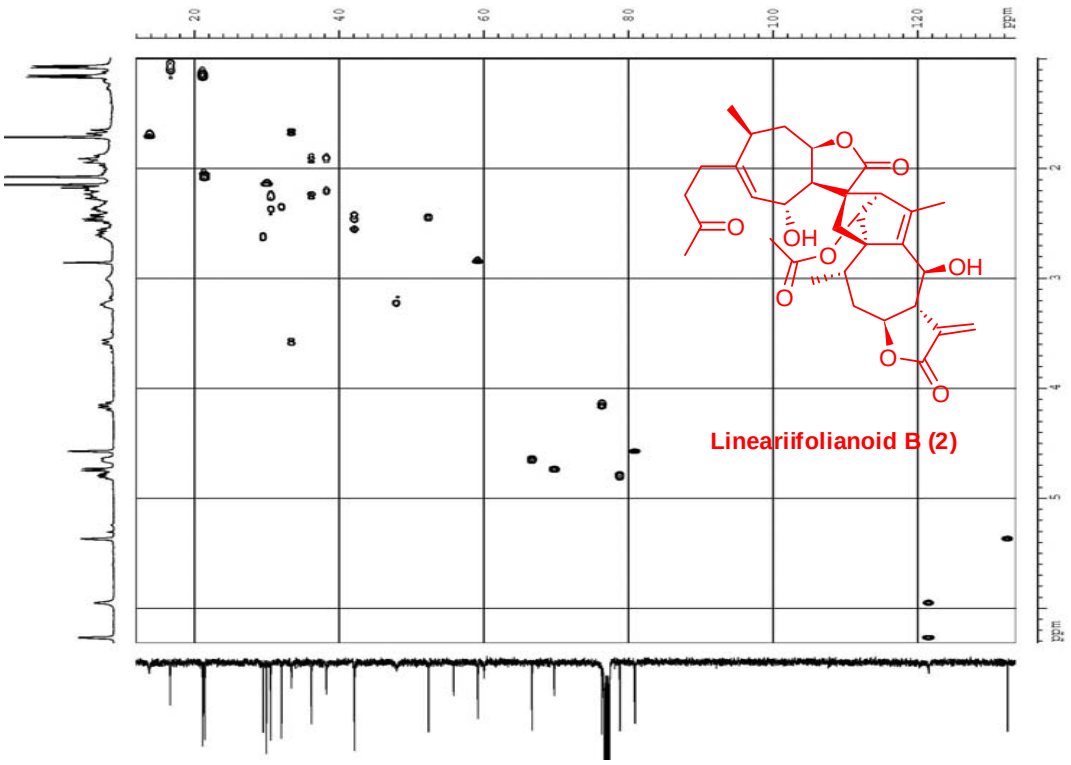

Fig. S18 ^{1}H–^{1}H COSY spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

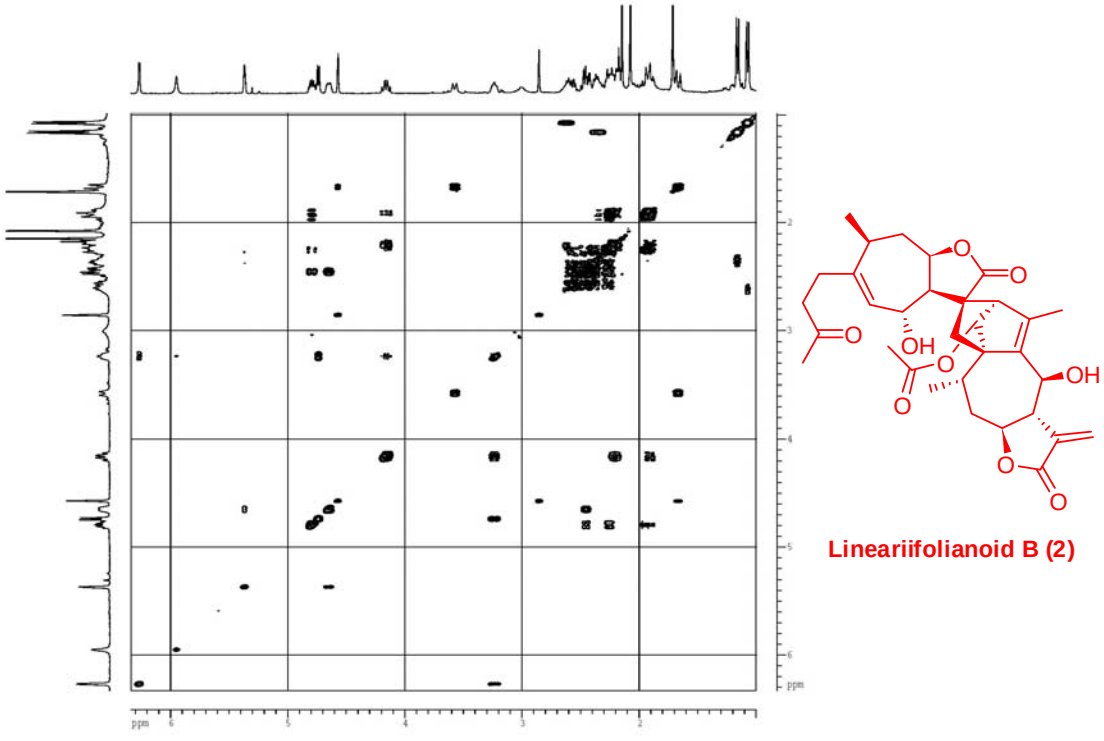

Fig. S19 HMBC spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

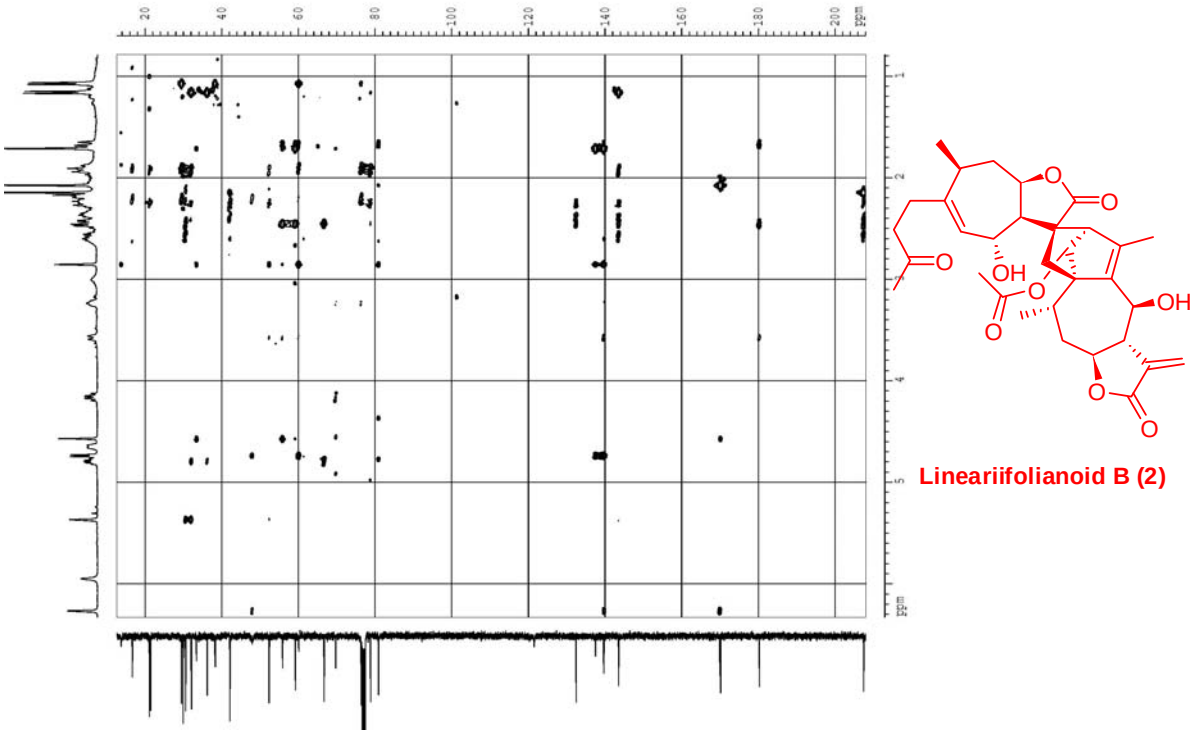

Fig. S20 NOESY spectrum of lineariifolianoid B (**2**) recorded in $CDCl_3$.

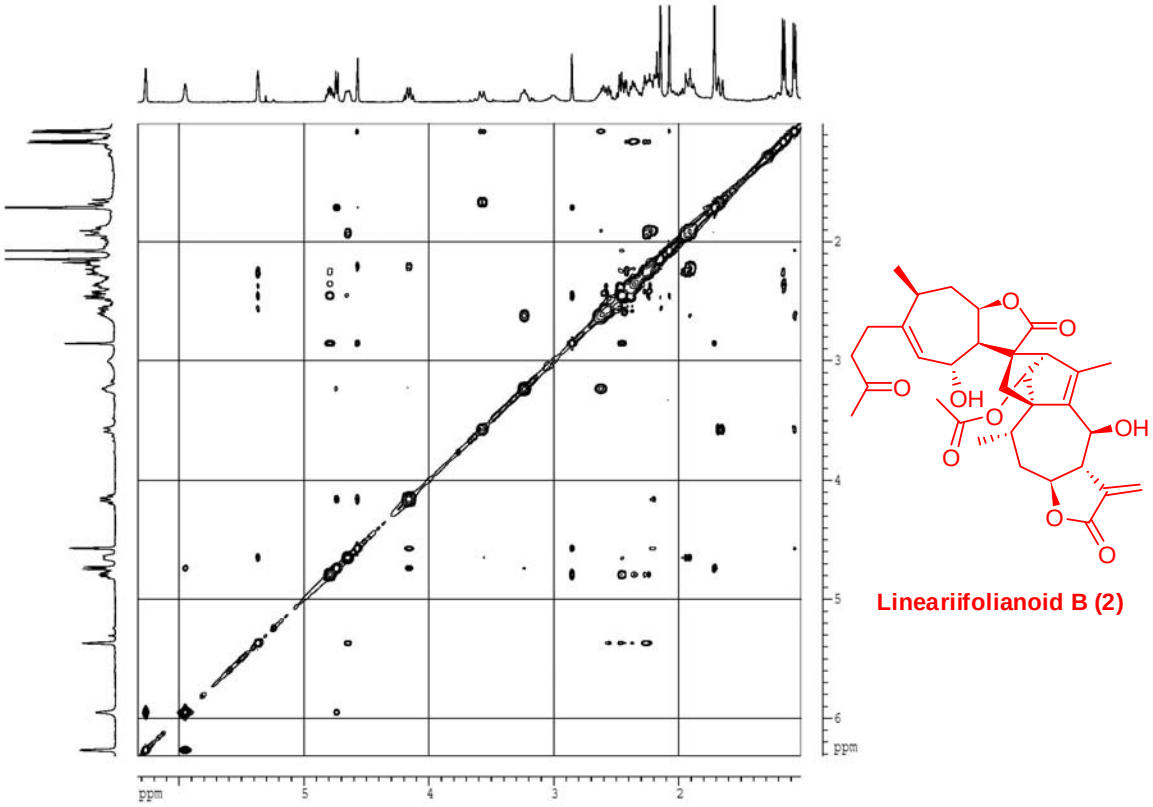

Fig. S21 ^{1}H NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

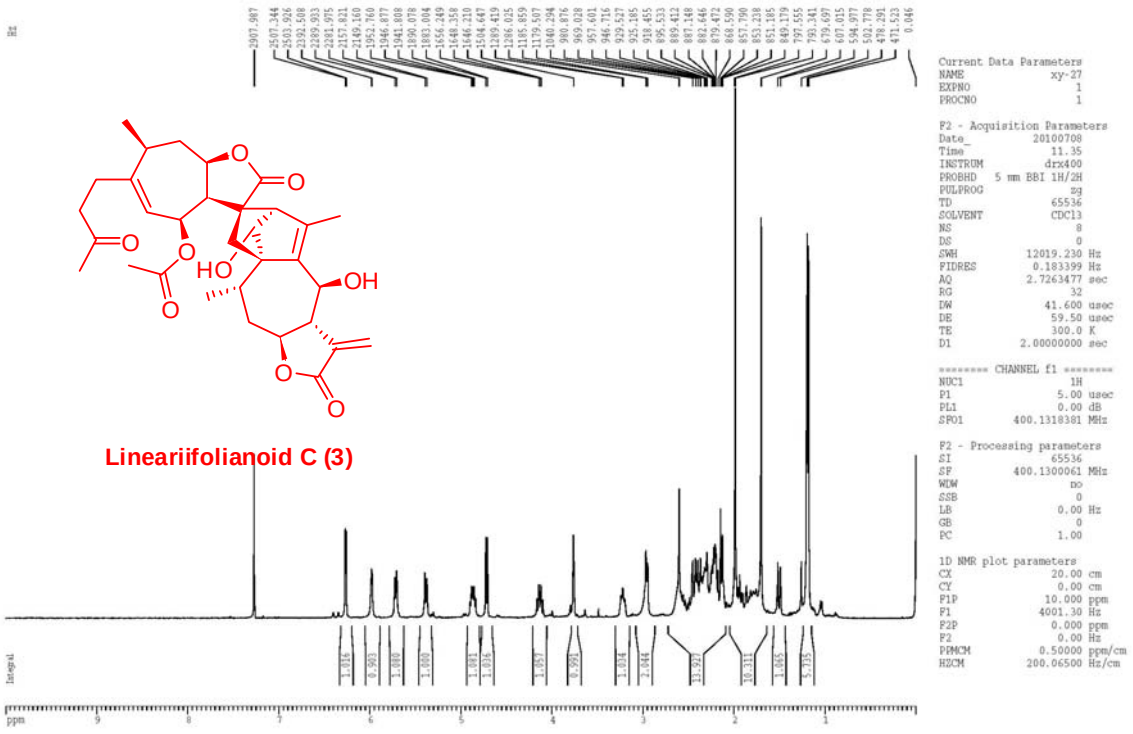

Fig. S22 ^{13}C NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

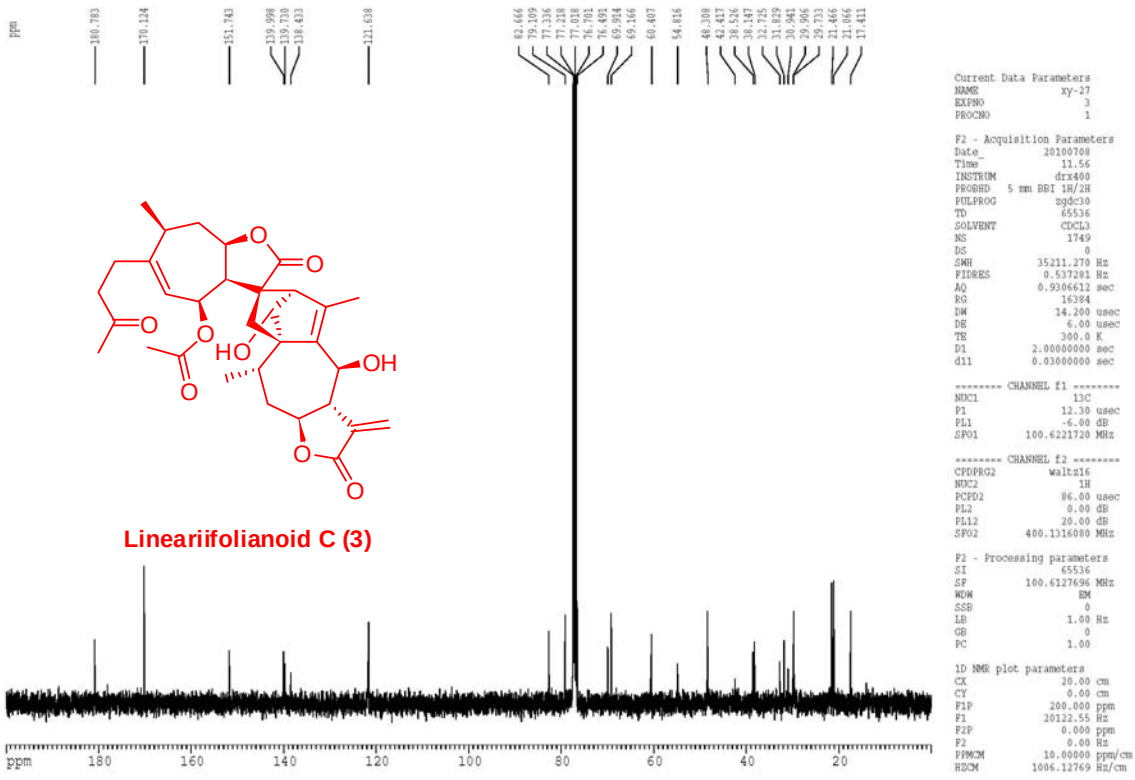

Fig. S23 DEPT NMR spectrum of lineariifolianoid C (**3**) recorded at 400 MHz in $CDCl_3$.

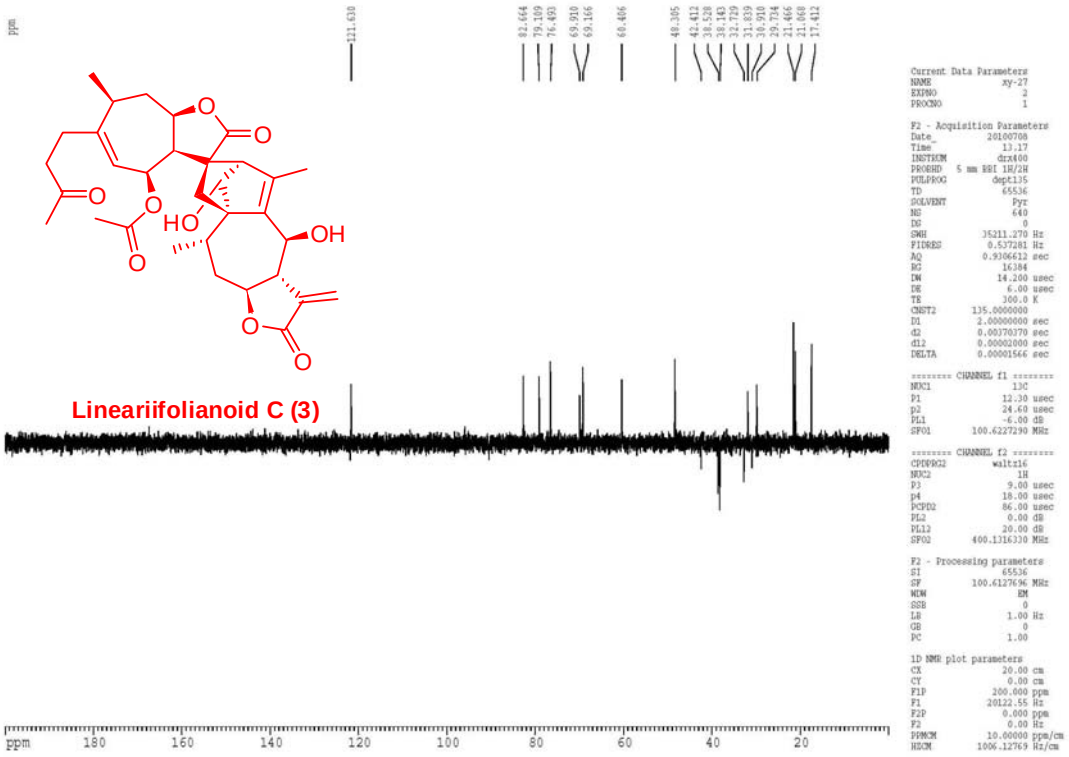

Fig. S24 HSQC spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

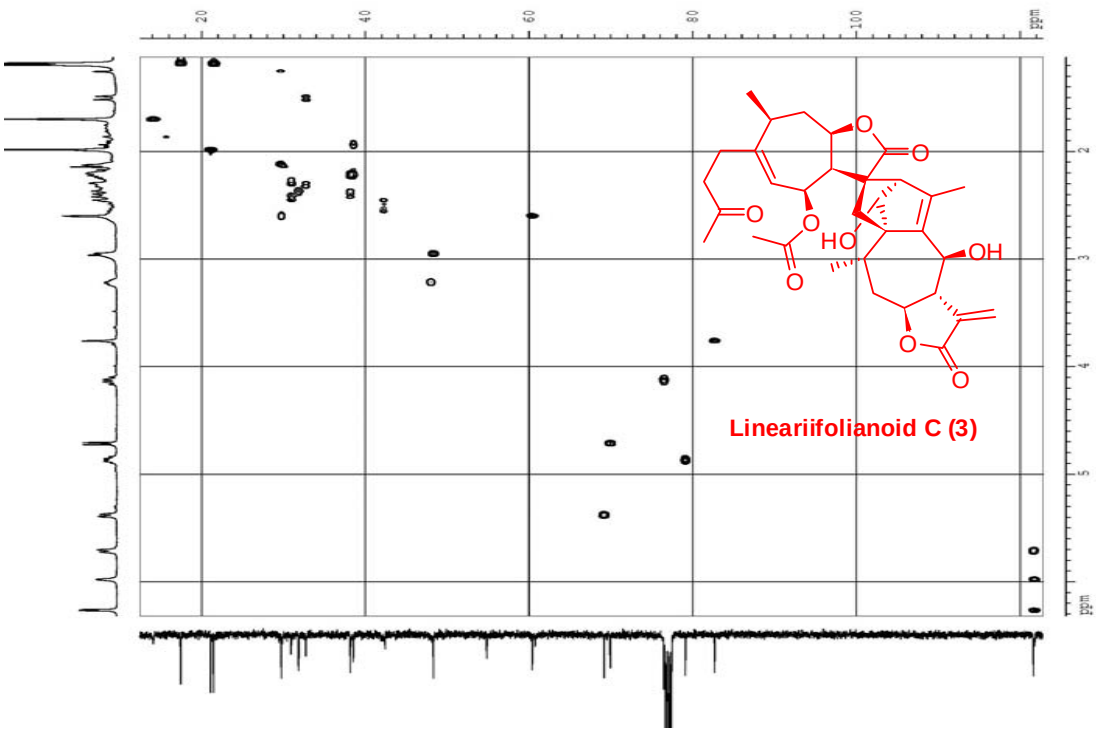

Fig. S25 $^{1}H–^{1}H$ COSY spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

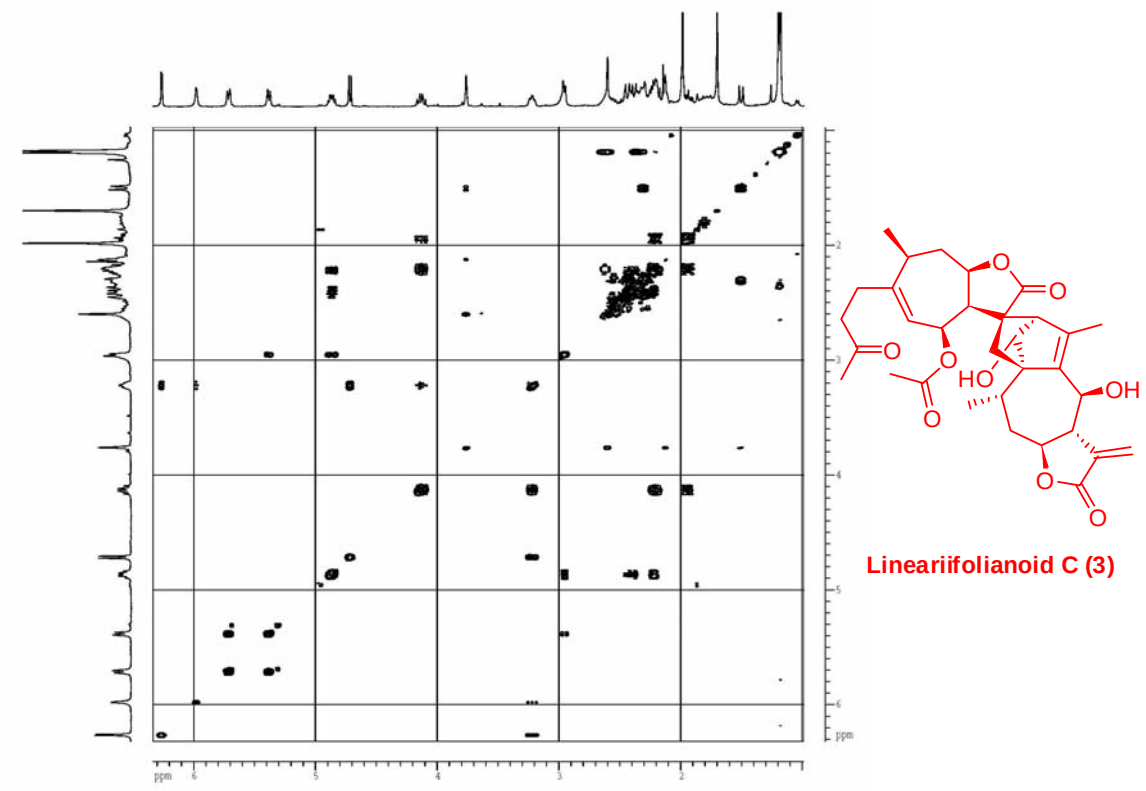

Fig. S26 HMBC spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

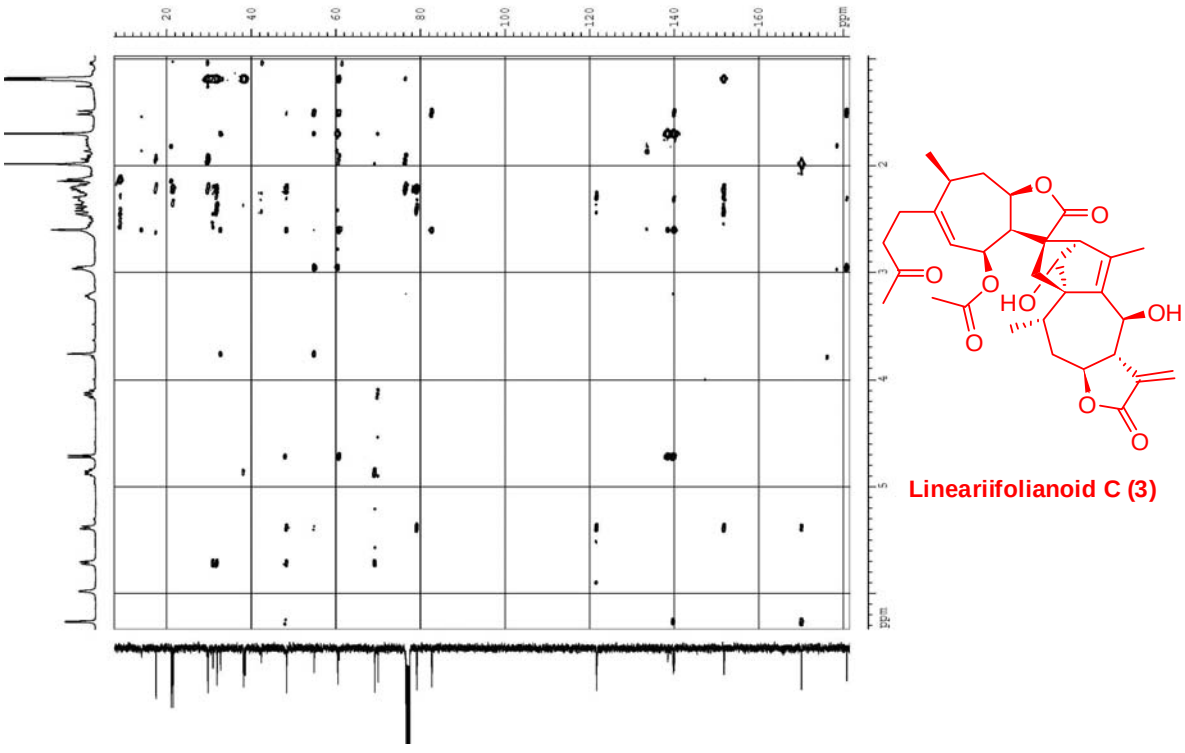

Fig. S27 NOESY spectrum of lineariifolianoid C (**3**) recorded in $CDCl_3$.

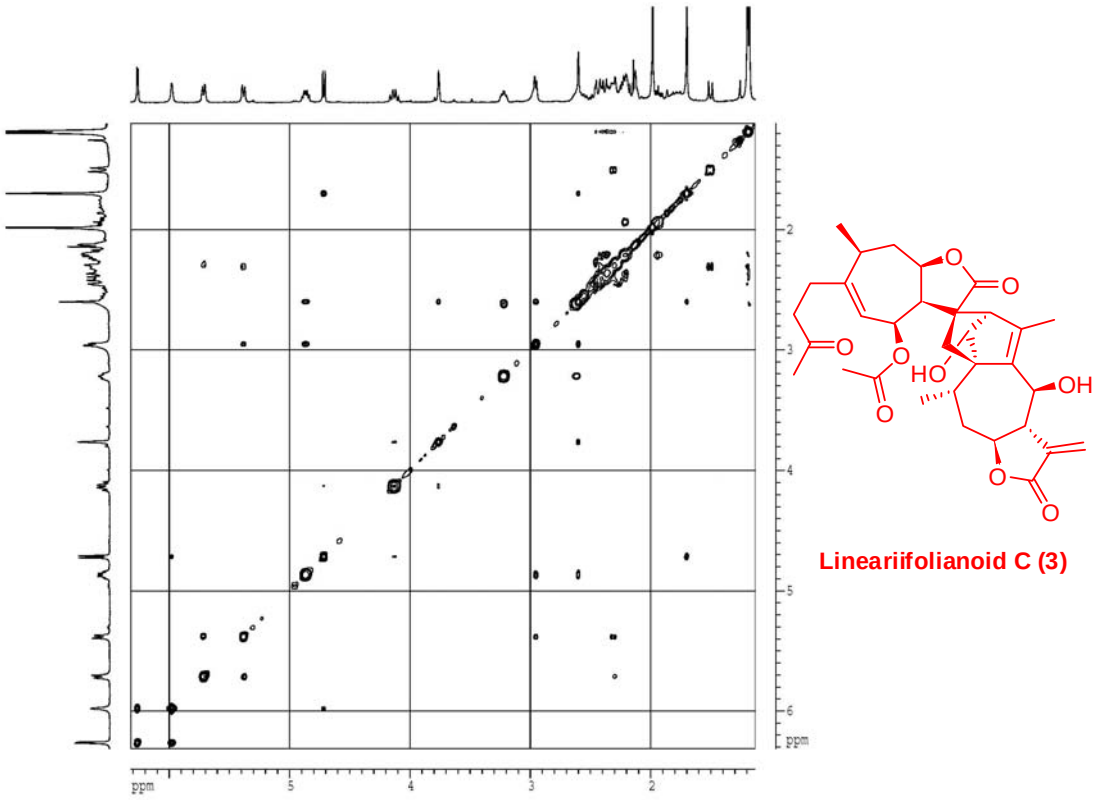

Fig. S28 ^1H NMR spectrum of lineariifolianoid D (4) recorded at 400 MHz in $CDCl_3$.

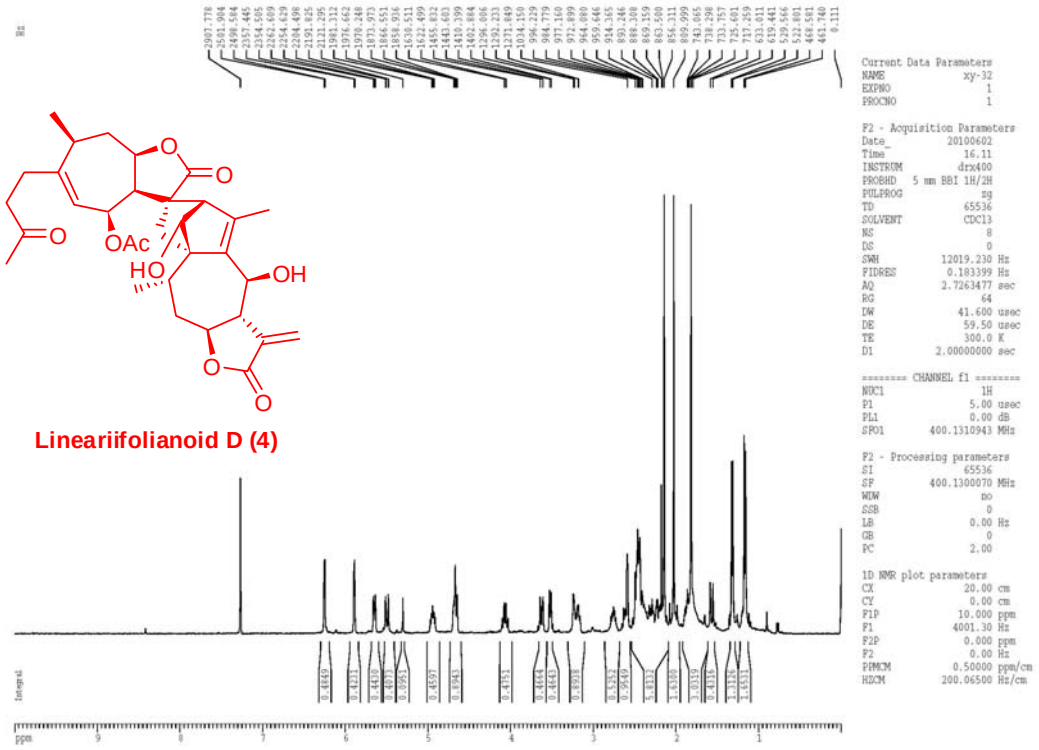

Fig. S29 ^{13}C NMR spectrum of lineariifolianoid D (**4**) recorded at 400 MHz in $CDCl_3$.

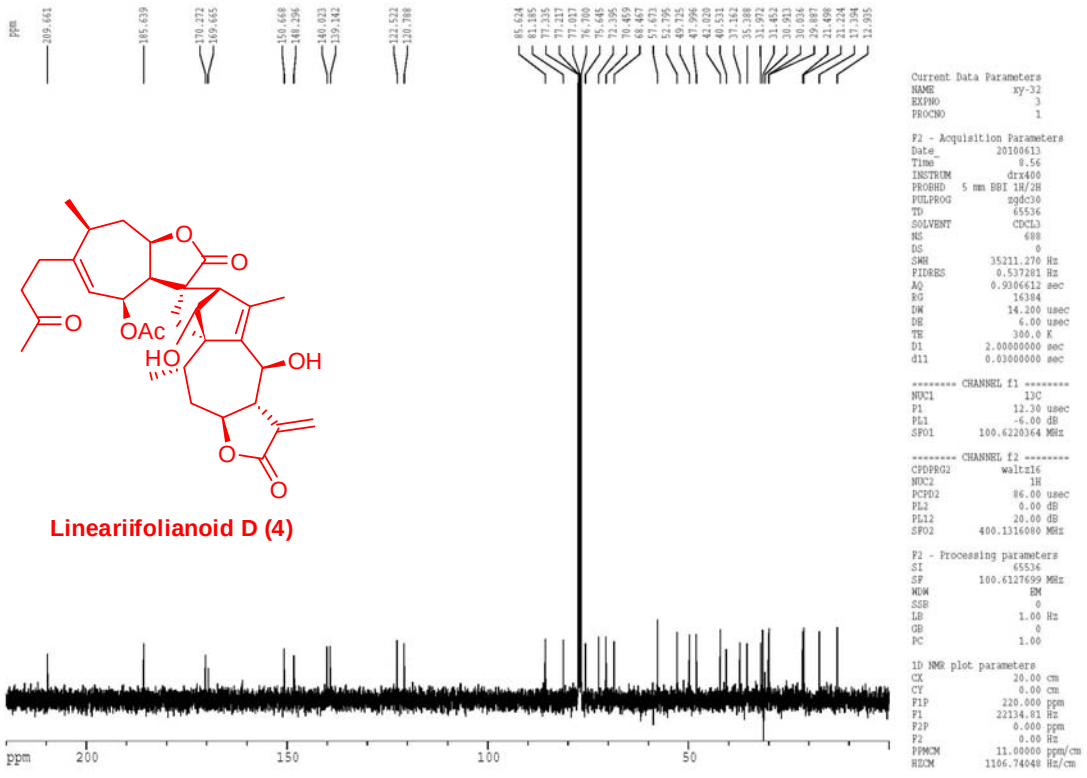

Fig. S30 DEPT NMR spectrum of lineariifolianoid D (**4**) recorded at 400 MHz in $CDCl_3$.

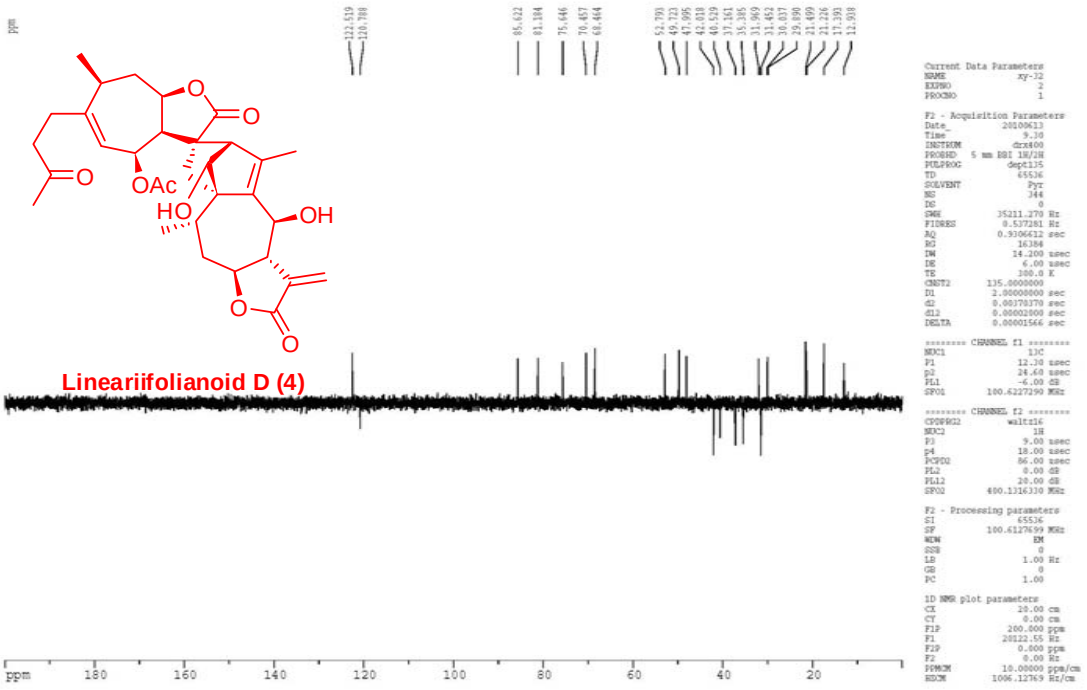

Fig. S31 HSQC spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

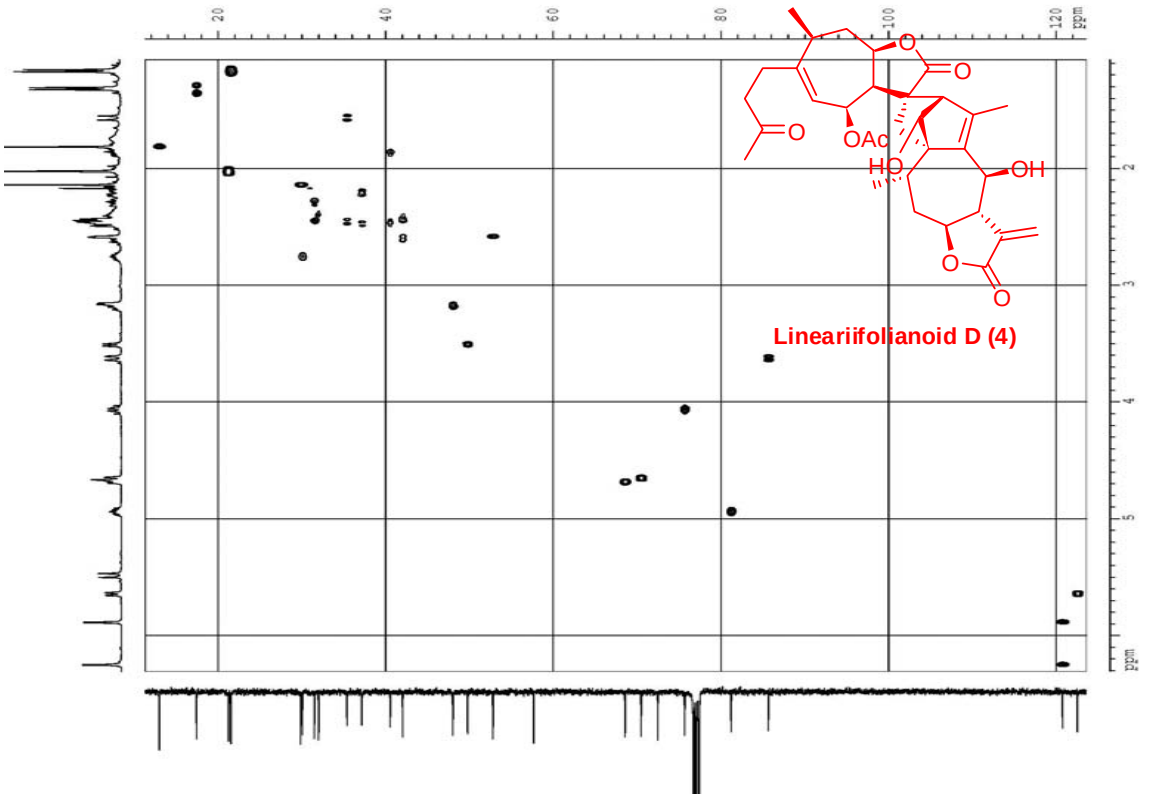

Figure S32. ^{1}H–^{1}H COSY spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

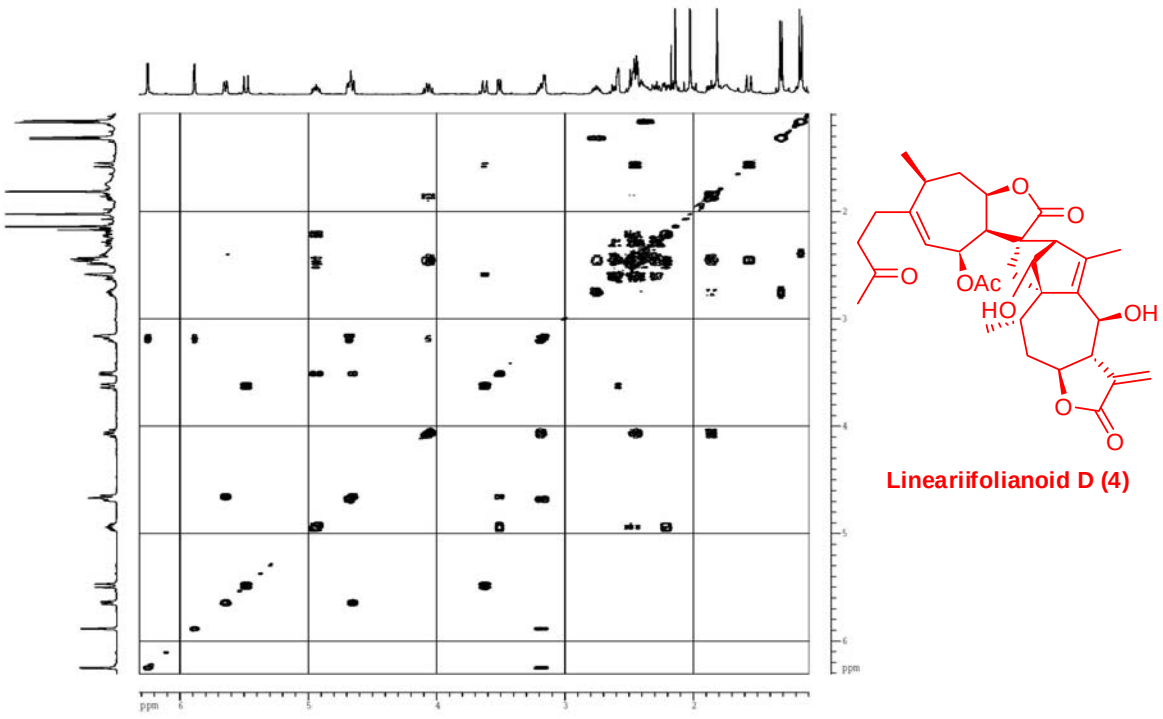

Fig. S33 HMBC spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.

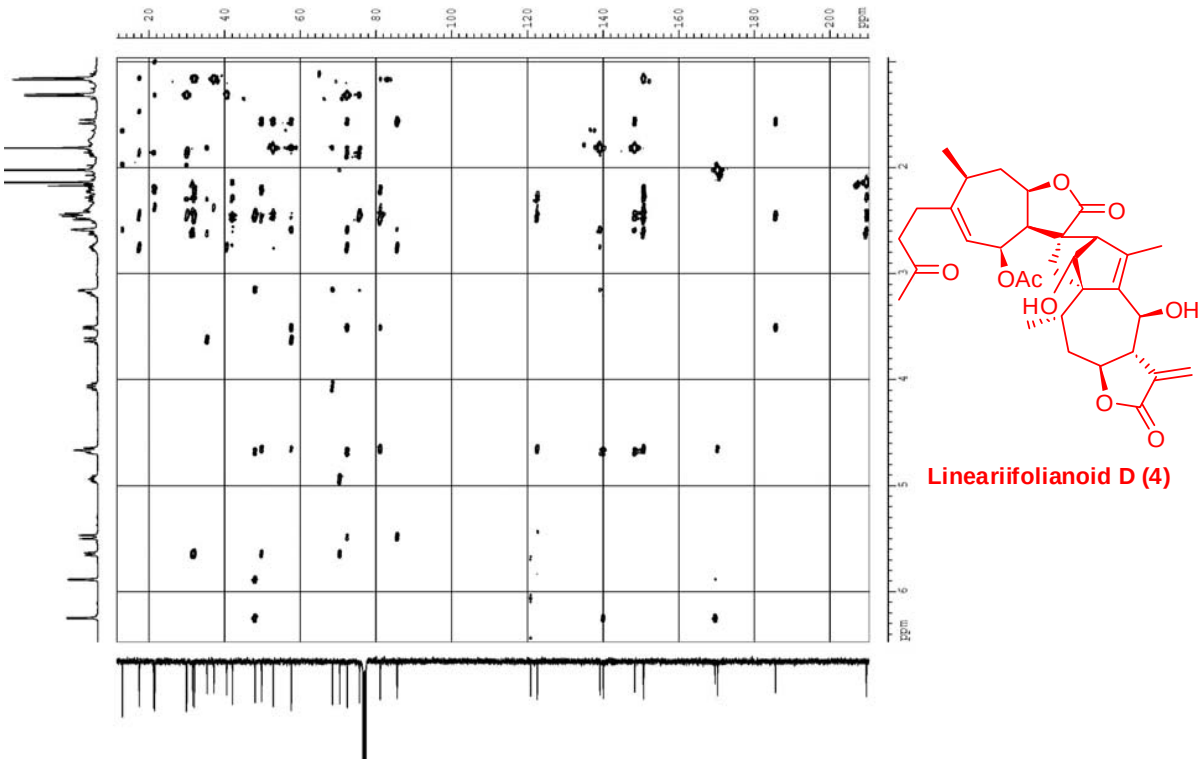

Fig. S34 NOESY spectrum of lineariifolianoid D (**4**) recorded in $CDCl_3$.