Supporting Information for:

Kalanchosides A–D, New Cytotoxic Bufadienolides from the Aerial Parts of

Kalanchoe gracilis

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General. Melting points were recorded on a Yanaco MP-3 melting point apparatus and are not corrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance 300 and AMX 400 FT-NMR spectrometers; all chemical shifts were given in ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer with a direct inlet system.

Plant Material. The aerial parts of *Kalanchoe gracilis* were purchased from a market (I-Shen galenic shop) in Taipei, Taiwan, in July 2003. It was authenticated by Professor C. S. Kuoh, Department of Biology, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No: PLW-0302) is deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried aerial parts of *K. gracilis* (5.5 kg) were extracted with MeOH under reflux (8×). The combined extracts were concentrated under reduced pressure to give a dark brown syrup. The syrup was then suspended in H₂O, defatted with hexane and then partitioned with CHCl₃ and EtOAc, successively. The concentrated CHCl₃ layer (14 g) was chromatographed on a silica gel column, eluting with a gradient of hexane-Me₂CO (4:1) to pure Me₂CO to yield ten fractions. Fraction 6 was subjected to repeated chromatography on silica gel to give **7** (5 mg). Fraction 7 was repeatedly chromatographed on a silica gel column to give **4** (10 mg), **6** (25 mg), and **8** (19 mg), successively. Fraction 8 was rechromatographed to give **3** (9 mg). The concentrated EtOAc layer (50 g) was subjected to column chromatography on Cosmosil 75 C18, eluted with a gradient of H₂O-MeOH (from pure H₂O to pure MeOH) to give nine fractions. Fraction 2 was subjected to chromatography on a silica gel column eluting with a gradient of CHCl₃-Me₂CO (1:2) to pure Me₂CO to give **1** (14 mg) and **2** (3 mg). Fraction 3 was further chromatographed on a silica gel column eluting with a gradient of CHCl₃-MeOH (5:1) to pure MeOH to give **5** (8 mg).

Kalanchoside A (1). White amorphous powder; $[\alpha]_D -41.9^\circ$ (c 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.0), 214 (3.9, sh), 295 (3.2) nm; IR (KBr) ν_{max} 3402, 1709, 1529 cm^{-1} ; FABMS m/z 585 $[\text{M} + \text{Na}]^+$; HR-FABMS m/z 563.2853 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{30}\text{H}_{43}\text{O}_{10}$ 563.2856). ^1H -NMR data, Table 1; ^{13}C -NMR data, Table 2.

Kalanchoside B (2). White amorphous powder; $[\alpha]_D -48.4^\circ$ (c 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.6), 222 (3.2, sh), 297 (2.8) nm; IR (KBr) ν_{max} 3418, 1713, 1538 cm^{-1} ; FABMS m/z 585 $[\text{M} + \text{Na}]^+$, 563 $[\text{M} + \text{H}]^+$; HR-FABMS m/z 563.2855 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{30}\text{H}_{43}\text{O}_{10}$ 563.2856). ^1H -NMR data, Table 1; ^{13}C -NMR data, Table 2.

Kalanchoside C (3). White amorphous powder; $[\alpha]_D -9.6^\circ$ (c 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.7), 296 (2.9) nm; IR (KBr) ν_{max} 3382, 1704, 1538 cm^{-1} ; FABMS m/z 561 $[\text{M} + \text{H}]^+$; HR-FABMS m/z 561.2702 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{30}\text{H}_{41}\text{O}_{10}$ 561.2700). ^1H -NMR data, Table 1; ^{13}C -NMR data, Table 2.

Kalanchoside D (4). White amorphous powder; $[\alpha]_D -107.6^\circ$ (c 0.49, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.4), 262 (2.7), 296 (2.5) nm; IR (KBr) ν_{max} 3365, 1709, 1538 cm^{-1} ; FABMS m/z 491 $[\text{M} + \text{H}]^+$; HR-FABMS m/z 491.2278 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{35}\text{O}_9$ 491.2281). ^1H -NMR data, Table 1; ^{13}C -NMR data, Table 2.

S5. Table 1. ¹H NMR data for **1-4**^a

position	1	2	3	4
H-1	1.66; 2.17	1.66; 2.14	1.65; 2.29	4.65 dd (12.6, 3.7)
H-2	1.78	1.72	1.76; 1.94	1.50 (α); 2.07 (β)
H-3	4.07 br s	4.05 br s	4.03 br s	3.81 br s
H-4	1.58; 2.10	1.60; 2.07	1.71; 2.06	1.89
H-6	1.61; 2.17	1.62; 2.18	1.71; 2.19	1.50 (β); 1.95 (α)
H-7	1.39; 2.25	1.34; 2.20	1.42; 2.29	1.18 (α); 1.84 (β)
H-8	1.96	1.95 td (12.4, 3.7)	2.46 td (12.6, 2.8)	2.50 td (11.5, 2.5)
H-9	1.78	1.75 td (12.4, 3.0)	1.96	1.29 t (11.5)
H-11	1.16; 1.58	1.16; 1.50	2.27dd (14.0, 4.2) 2.59 t (14.0)	3.99 td (11.5, 2.6)
H-12	1.35; 1.45	1.40; 1.48		1.46 (α); 2.04 (β)
H-15	1.64; 2.10	1.66; 2.10	1.42; 1.71	1.56 (β); 2.14 (α)
H-16	1.76; 2.07	1.69; 2.07	1.71; 1.90	1.84 (β); 2.18 (α)
H-17	2.54 dd (9.0, 6.5)	2.53 dd (9.7, 6.0)	3.95 m	2.69 t (8.1)
H-18	0.69 s	0.69 s	0.88 s	0.86 s
H-19	10.07 s	10.07 s	10.05 s	5.79 d (4.8)
H-21	7.40 d (1.6)	7.39 d (2.2)	7.44 d (1.5)	7.41 d (2.5)
H-22	7.95 dd (9.8, 1.6)	7.95 dd (9.7, 2.2)	7.85 dd (9.8, 1.5)	7.83 dd (9.7, 2.5)
H-23	6.17 d (9.8)	6.17 d (9.7)	6.20 d (9.8)	6.20 d (9.7)
H-1'	4.87 d (3.7)	4.88 d (3.2)	4.85 d (3.9)	
H-2'	3.44 dd (9.3, 3.7)	3.76 m	3.53 dt (8.7, 3.9)	
H-3'	3.60 t (9.3)	3.76 m	3.95 m	
H-4'	2.99 t (9.3)	3.68 br s	1.51(ax), 1.83(eq) m	
H-5'	3.63 dq (9.3, 6.3)	3.97 br q (6.4)	4.25 m	
H-6'	1.18 d (6.3)	1.18 d (6.4)	1.11 d (6.3)	
OH-2'	3.62 brs	3.57 br s	3.88 d (8.7)	
OH-3'	- ^b	- ^b	3.74 d (5.4)	
OH-4'	4.08 br s	- ^b		
OH-3				3.94 s
OH-5	4.68 s	4.70 s	4.81 s	5.03 s
OH-14	3.43 s	3.45 s	4.15 s	3.52 s
OH-19				6.59 s
OAc				2.02 s

^a δ, multiplicity, *J*, Hz in parentheses, all compounds measured in acetone-d₆^b The chemical shift of OH may overlap with those of sugar's protons

S6. Table 2. ^{13}C NMR data for **1-4**^a

Position	1	2	3	4
C-1	18.8	18.9	19.2	73.0
C-2	26.2	26.1	25.7	39.5
C-3	72.1	71.9	75.7	65.3
C-4	37.9	38.0	37.8	47.6
C-5	73.5	73.5	73.8	76.2
C-6	34.7	34.7	35.8	35.1
C-7	25.1	25.2	25.2	20.5
C-8	42.8	42.8	41.7	39.7
C-9	40.0	40.0	36.6	49.5
C-10	55.7	55.7	55.0	54.5
C-11	23.2	23.2	38.3	79.9
C-12	41.0	41.1	211.8	47.9
C-13	48.9	48.9	64.0	52.7
C-14	84.9	85.0	85.6	86.0
C-15	32.3	32.4	32.2	32.2
C-16	29.3	29.3	29.5	31.0
C-17	51.6	51.6	40.6	51.4
C-18	16.9	16.8	17.4	20.5
C-19	208.5	208.5	209.1	104.8
C-20	123.4	123.4	122.1	122.8
C-21	150.0	150.0	150.9	149.9
C-22	147.8	147.7	147.7	147.5
C-23	115.3	115.4	115.7	115.5
C-24	162.0	162.0	162.0	161.9
C-1'	97.3	97.7	100.7	
C-2'	73.0	69.3	68.7	
C-3'	74.7	71.2	68.3	
C-4'	76.9	72.8	40.4	
C-5'	69.0	67.5	60.3	
C-6'	18.2	16.9	21.0	
OAc				21.0, 170.9

^a All compounds measured in acetone- d_6

S7. ^1H NMR spectrum of kalanchoside A (**1**) in acetone- d_6

S18. ^{13}C NMR spectrum of kalanchoside A (**1**) in acetone- d_6

S19. ^1H NMR spectrum of kalanchoside B (**2**) in acetone- d_6

S10. ^{13}C NMR spectrum of kalanchoside B (**2**) in acetone- d_6

S11. ^1H NMR spectrum of kalanchoside C (**3**) in acetone- d_6

S12. ^{13}C NMR spectrum of kalanchoside C (**3**) in acetone- d_6

S13. ^1H NMR spectrum of kalanchoside D (**4**) in acetone- d_6

S14. ^{13}C NMR spectrum of kalanchoside D (**4**) in acetone- d_6