New Cassane-Type Diterpenoids from *Caesalpinia bonduc*

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Six new cassane diterpenoids, named caesalls H–M (1–6), were isolated from the seed kernels of *Caesalpinia bonduc*. Their structures were elucidated on the basis of spectroscopic analysis, mainly NMR and MS. The absolute configurations of compounds 1 and 3 were determined by a single-crystal X-ray study using a mirror Cu*Kα* radiation and circular dichroism (CD) spectra, respectively. None of the compounds were cytotoxic against HepG-2, MCF-7 and MG-63 cells.

Key words *Caesalpinia bonduc*; cassane-type diterpenoid; single-crystal

Caesalpinia bonduc (Linn.) Roxb. (Fabaceae), a rich source of cassane-type diterpenoids, is a stout prickly climber distributed throughout the tropical and subtropical regions. The structures of these diterpenoids are characterized by a molecular skeleton constructed of three fused cyclohexane rings and a furan ring or an *α*,*β*-butenolide moiety. Some cassane-type diterpenoids are known to possess antitumor, antimalarial, antibacterial, antihelmintic, and antineoplastic properties.[1–5] Previous phytochemical investigations on plants of *Caesalpinia* have resulted in the isolation of several cassane and norcassane furanoditerpenes,[6–9] and seven new compounds, caesalls A–G,[10] were isolated in our previous research on *C. bonduc*. As a continuation of our study on this plant, the ethanol (95%) extract of the seed kernel was investigated. As a result, six new compounds (**1**–**6**) were obtained. Their structures were elucidated by extensive one dimensional (1D) and 2D NMR (heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC), and rotating frame Overhauser enhancement spectroscopy (ROESY)) and mass (high resolution-electrospray ionization-mass spectrum (HR-ESI-MS)) spectroscopic data analysis. The absolute configuration of compound **1** was determined by a single-crystal X-ray diffraction experiment, and which of compound **3** was determined by circular dichroism (CD) spectra. None of the compounds were cytotoxic against HepG-2, MCF-7 and MG-63 cells. Herein, we reported the isolation and structure elucidation of compounds **1**–**6**.

Results and Discussion

Compound **1** was recrystallized from CH_2Cl_2–MeOH to yield colorless crystals. A quasi-molecular peak $[M+Na]^+$ at *m*/*z* 395.1830 in an HR-ESI-MS represents $C_{22}H_{28}O_5Na$ corresponded to a molecular formula of $C_{22}H_{28}O_5$. The ^{1}H-NMR spectrum showed signals corresponding to four tertiary methyls at δ_H 2.36 (H_3-17), 1.28 (H_3-18), 1.17 (H_3-19) and δ_H 1.34 (H_3-20), two oxygen-substituted methines at δ_H 4.56 (H-1), 5.71 (H-6), together with two protons of a 1,2-disubstituted furan ring (δ_H 7.55, d, *J*=2.0 and δ_H 6.74, d, *J*=2.0). In addition, the trisubstituted benzofuran moiety resonated the downfield signals at δ_H 7.55 (H-16), 6.74 (H-15), 7.40 (H-11), and one aromatic methyl group at δ_H 2.36 (H_3-17). The 1D NMR data of compound **1** was closely related to these of 6-acetoxy-3-deacetoxycaesaldekarin e,[2] except for the acetyl group at C-1 in 6-acetoxy-3-deacetoxycaesaldekarine that was replaced by hydroxyl group in **1**. The locations of 6-OAc and 1-OH were deduced from HMBC correlations observed from a proton signal at δ_H 5.71 (H-6) to –O$\underline{C}$OCH$_3$ (δ_C 170.5) and C-5 (δ_C 78.2), and H_3-20 (δ_H 1.34, s) to C-1 (δ_C 73.5), H-1 (δ_H 4.56, br s) to C-10 (δ_C 49.4). The relative stereochemistry of **1** was determined by ROESY spectral analysis. The methyl at δ_H 1.34 (H_3-20) showed cross peaks with H-1 and H-6 indicated that the acetoxy group at C-6 and the hydroxyl group at C-1 were *α*-oriented. The absolute configuration of **1** was determined by X-ray diffraction study as shown in Fig. 2. Thus, the structure was confirmed and the absolute configuration of **1** was finally determined to be 1*S*,5*R*,6*S*,10*S* and named caesall H.

Compound **2** had a molecular formula $C_{22}H_{26}O_5$, based on HR-ESI-MS ($[M+H]^+$ at *m*/*z* 371.1850) which was 2 mass units less than that of **1**. A comparison of the NMR data (Table 1) of compounds **1** and **2** showed that the hydroxyl group at C-1 in **1** was replaced by ketone carbonyls in **2.** This difference was confirmed by the carbon signal at δ_C 211.0 and the HMBC correlation of H_3-20 (δ_H 1.74, s) with C-1 (δ_C 211.0). In the ROESY spectrum, correlations from H-6 to H_3-20 and H_3-19 indicated that the acetoxy group at C-6 was *α*-oriented. Caesall I (**2**) is, therefore, the 1-keto analogue of **1** and a perspective view of the molecular structure is presented in Fig. 1.

Compound **3**, showed $[M+Na]^+$ ion peak at *m*/*z* 543.2204 (calcd for $C_{27}H_{36}O_{10}Na$ 543.2201) and exhibited hydroxyl and carbonyl groups at 3455, 1749 cm^{-1} in the IR spectra. In the ^{1}H- and ^{13}C-NMR spectrum, the olefinic proton signal at δ_H 5.90 (H-15, s) and carbon signals at δ_C 107.6 (C-12), 115.3 (C-15), 166.3 (C-13) and 169.8 (C-16) suggested the presence of the *α*,*β*-butenolide moiety. The HMBC correlations (Fig. 3) of H-15 (δ_H 5.90, s) with C-13, C-16 confirmed the presence of such a moiety, combined with the three methyl signals at δ_H 1.12 (H_3-18, s), 1.17(H_3-20, s), 1.21(H_3-19, s), which are typical signals for cassane-type diterpenes, that indicated **3** was a tetracyclic cassane diterpene possessing a fuesd butenolide unit. The obtained ^{1}H- and ^{13}C-NMR data were similar to caesalpin G,[8] except for the absence of one hydroxyl group, the presence of two acetoxy groups and the difference of the relative configuration at C-12. Three acetoxy groups were attached at C-1, C-2, C-3 respectively, on the basis of the HMBC correlations from H-1 (δ_H 5.22, d, *J*=3.0) to –O$\underline{C}$OCH$_3$ (δ_C 169.4), from H-2 (δ_H 5.50, t, *J*=3.0) to –O$\underline{C}$OCH$_3$ (δ_C 169.9),

The authors declare no conflict of interest.

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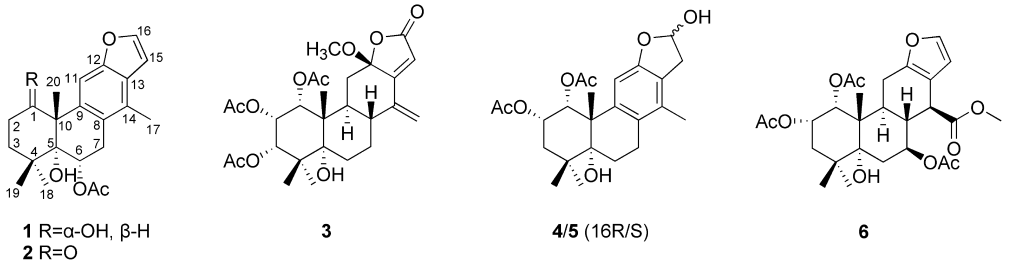

Fig. 1. Compounds from the Seed Kernels of *C. bonduc*

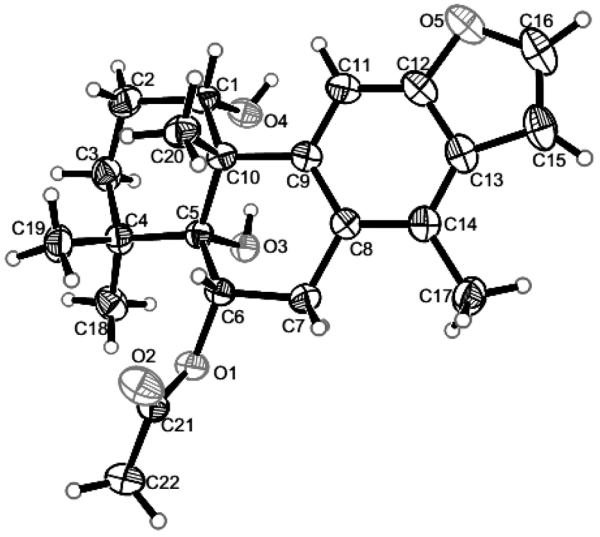

Fig. 2. Single-Crystal X-Ray of Compound **1**

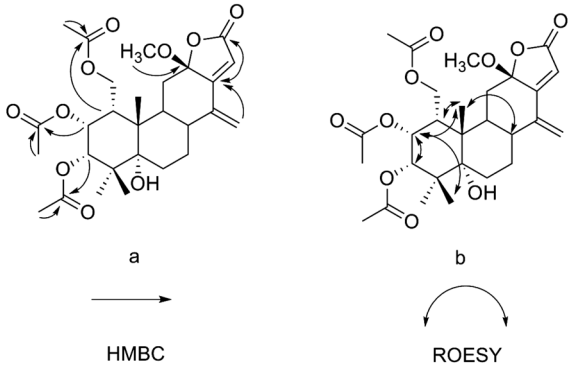

Fig. 3. Key HMBC and ROESY of Compound **3**

from H-3 (δ_H 5.15, d, J=3.0) to –OCOCH$_3$ (δ_C 169.4). The ROESY (Fig. 3) correlations of H$_3$-20 with H-1, H-2 and H-8; H-2 with H-3 and H$_3$-19 indicated that the acetoxy groups at C-1, C-2 and C-3 were α-oriented. Furthermore, the proton signal at δ_H 2.83 (H-9) showed no cross peaks with H$_3$-20 and 12-OCH$_3$ in ROESY spectrum indicated that H-9 was α-oriented and 12-OCH$_3$ was β-oriented. The CD spectrum of **3** displayed a positive Cotton effect at 260 nm and a positive Cotton effect at 219 nm, confirming the (8*R*, 12*S*) absolute configuration for **3** based on the exciton chirality method and the γ-lactone rules.[8,11–13] Thus, the structure of **3** was established as1α,2α,3α-triacetoxy-5α-hydroxy-12β-methoxycassa-14(17),13(15)-dien-16,12-olide and named caesall J.

Compounds **4**/**5** were isolated as a diasteromixture at C-16 position. The molecular formula of **4**/**5** was determined to be $C_{24}H_{32}O_7$ by HR-ESI-MS (m/z 455.2043 [M+Na]$^+$). The ^{1}H-NMR spectrum (Table 1) of **4**/**5** indicated that it was an approximately 1 : 1 mixture of two stereoisomers, which we were unable to separate. Despite this, the low-field chemical shifts of H-11 (δ_H 6.44, s and 6.43, s), C-8 (δ_C 126.7, overlapping), C-9 (δ_C 142.4, overlapping), C-11 (δ_C 102.9 and 102.8), and C-14 (δ_C 133.3, overlapping) suggested that ring C in **4**/**5** is aromatic. The ^{1}H-NMR spectrum displayed three methyl signals at δ_H 1.16 (H$_3$-18, s), 1.20 (H$_3$-19, s) and 1.41 (H$_3$-20, s), two acetoxy methyl signals at δ_H 2.02, 1.98/1.97 and three oxymethines signals at δ_H 5.86 (H-1, s), 5.46/5.47 (H-2, d, J=13.0, 3.0) and 6.02/6.01 (H-16, d, J=7.0, 2.0). These data indicated that **4** was a tetracyclic cassane diterpene with two acetoxy substituents. Lack of fused furan ring signals and the presence of a oxymethine signal at δ_H 6.01/6.02, two protons signs at δ_H 2.95, 3.27 indicated the presence of a dihydrofuran ring. The location of H-16 was deduced from the HMBC correlations of H-16 (δ_H 6.01/6.02) with C-15 (δ_C 37.4), C-12 (δ_C 156.0), and C-13 (δ_C 122.2). The HMBC correlations (Fig. 4) of –OCOCH$_3$ (δ_H 1.98, s and 1.99, s) with C-1 (δ_C 74.5, overlapping) and –OCOCH$_3$ (δ_C 169.5, 169.7) and of –OCOCH$_3$ (δ_H 2.02, overlapping) with C-2 (δ_C 68.0, overlapping) and –OCOCH$_3$ (δ_C 170.6) indicated that the acetoxy groups were attached to C-1 and C-2. In the ROESY spectra, the methyl at δ_H 1.41 (H$_3$-20) showed cross peaks with H-1 and H-2 indicated the acetyl substituents at C-1 and C-2 to be α-axially oriented. Therefore, the structures of **4**/**5** were determined and named caesall K/L.

Compound **6** had the molecular formula $C_{27}H_{36}O_{10}$ determined by HR-ESI-MS. The ^{1}H-NMR spectrum of **6** displayed signals corresponding to three tertiary methyls, three oxygen-substituted methines, three acetyl methyls, and a methoxy. Moreover, the ^{13}C-NMR spectrum of **6** showed four olefinic carbons (δ_C 108.4, 113.4, 141.7, 149.8) and four oxygen-substituted carbons (δ_C 67.3, 74.5, 75.9, 78.0) together with four ester carbonyl carbons (δ_C 169.1, 170.2, 170.4, 174.6). The 1D NMR data (Table 1) of **6** were similar to those of caesalpinin MG,[14] except for the presence of an acetoxy group at C-2 and the absence of the acetoxy group at C-6. The location of 2-OAc was confirmed by the HMBC relations (Fig. 5) of 1-H (δ_H 5.28, brs) with C-2 (δ_C 67.3) and of –COOCH$_3$ (δ_H 1.97, s) with C-2 (δ_C 67.3) and –COOCH$_3$ (δ_C 170.4). The relative configuration of **6** was assigned by the ROESY spectrum, the correlation of H$_3$ −20 (δ_H 1.33, s) with H-1 (δ_H 5.28, brs) and H-2 (δ_H 5.32, ddd, J=13.0, 4.5, 3.0), of H-14 (δ_H 3.38, d, J=9.0) with H-7 (δ_H 5.20, td, J=11.0, 5.5) and H-9 (δ_H 2.61, td, J=12.0, 5.5) indicated that the acetoxyl substituents at C-1 and C-2 to be in α-oriented, and the acetoxyl substituents at C-7, C-14 to be β-oriented. Thus, the structure of caesall M was

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compounds **1**−**6** in $CDCl_3$

Position	**1**		**2**		**3**		**4**		**5**		**6**	
	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C
1	4.56, brs	73.5		211.0	5.22, d (3.0)	73.2	5.86, s	74.5	5.86, s	74.5	5.28, brs	74.5
2	1.93, m	24.9	2.79, m	36.5	5.50, t (3.0)	65.9	5.47, dd (13.0, 3.0)	68.0	5.46, dd (13.0, 3.0)	68.0	5.32, ddd (13.0, 4.5, 3.0)	67.3
	2.16, m											
3	1.21, m	32.5	1.97, m	39.0	5.15, d (3.0)	77.3	2.19, dd (13.0, 5.0)	36.1	2.16, dd (13.0, 5.0)	36.1	1.39, dd (13.5, 4.5)	35.4
	2.16, m						1.43, m		1.43, m		1.96, m	
4		38.6		38.2		43.1		40.0		40.0		40.2
5		78.2		79.9		76.5		75.6		75.6		78.0
6	5.71, dd (9.0, 7.5)	72.5	5.64, dd (9.5, 7.0)	72.2	1.77, m	26.2	1.99, m	24.0	1.99, m	23.9	1.60, dd (13.0, 5.5)	32.0
					1.68, m		2.03, m		2.03, m		2.16, dd (13.0, 5.5)	
7	2.86, dd (16.0, 9.0)	31.9	2.80, m	31.0	1.87, m	23.0	2.65, dd (17.0, 7.0)	23.7	2.65, dd (17.0, 7.0)	23.6	5.20, td (11.0, 5.5)	75.9
	3.29, dd (16.0, 7.5)		3.28, dd (16.0, 7.5)				2.76, dd (17.0, 8.0)		2.76, dd (17.0, 8.0)			
8		127.3		124.5	2.09, m	39.8		126.7		126.7	2.47, m	38.9
9		139.6		134.6	2.83, td (12.0, 2.5)	37.9		142.4		142.4	2.61,td (12.0, 5.5)	37.1
10		49.4		60.0		45.5		48.4		48.4		45.1
11	7.40, s	104.5	7.74, s	111.3	1.43, m	35.6	6.44, s	102.9	6.43, s	102.8	2.37, dd (16.0, 5.0)	21.6
					2.08, m						2.50, m	
12		154.1		153.5		107.6		156.0		156.0		149.8
13		126.1		126.8		166.3		122.3		122.2		113.4
14		128.9		128.0		143.6		133.3		133.3	3.38, d (9.0)	46.1
15	6.74, d (2.0)	105.2	6.71, d (2.0)	105.0	5.90, s	115.3	2.95, m	37.4	2.95, m	37.4	6.10, d (1.5)	108.4
							3.27, m		3.27, m			
16	7.55, d (2.0)	144.7	7.56, d (2.0)	145.3		169.8	6.01, dd (7.0, 2.0)	100.7	6.02, dd (7.0, 2.0)	100.6	7.22, d (1.5)	141.7
17	2.36, s	16.0	2.20, s	16.2	5.21, d (2.0)	113.6	2.12, s	16.4	2.12, s	16.4		174.6
					5.30, d (2.0)							
18	1.28, s	30.8	1.36, s	29.0	1.12, s	23.3	1.16, s	28.0	1.16, s	28.0	1.11, s	28.2
19	1.17, s	25.0	1.28, s	26.6	1.21, s	25.0	1.20, s	25.7	1.20, s	25.7	1.16, s	25.9
20	1.34, s	29.5	1.74, s	28.8	1.17, s	17.8	1.41, s	30.2	1.41, s	30.1	1.33, s	17.7
1-O$\underline{C}$OCH$_3$						169.4		169.5		169.7		169.1
1-OCO$\underline{C}$H$_3$					2.11, s	20.9	1.99, s	21.1	1.98, s	21.1	2.12, s	21.2
2-O$\underline{C}$OCH$_3$						169.9		170.6		170.6		170.4
2-OCO$\underline{C}$H$_3$					1.96, s	20.7	2.02, s	21.2	2.02, s	21.2	1.97, s	21.0
3-O$\underline{C}$OCH$_3$						169.4						
3-OCO$\underline{C}$H$_3$					2.10, s	20.8						
6-O$\underline{C}$OCH$_3$		170.5		170.3								
6-OCO$\underline{C}$H$_3$	2.18, s	22.0	2.09, s	21.9								
7-O$\underline{C}$OCH$_3$												170.2
7-OCO$\underline{C}$H$_3$											1.98, s	21.0
12-OCH$_3$					3.08, s	50.2						
17-OCH$_3$											3.71, s	52.0

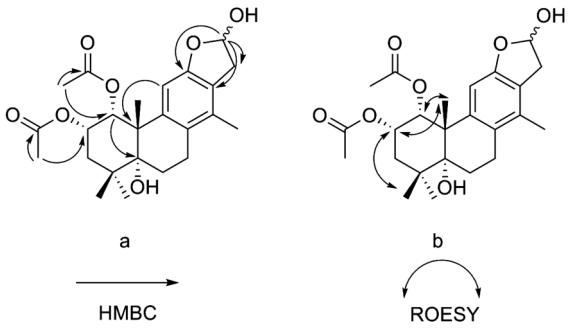

Fig. 4. Key HMBC and ROESY of Compound **4/5**

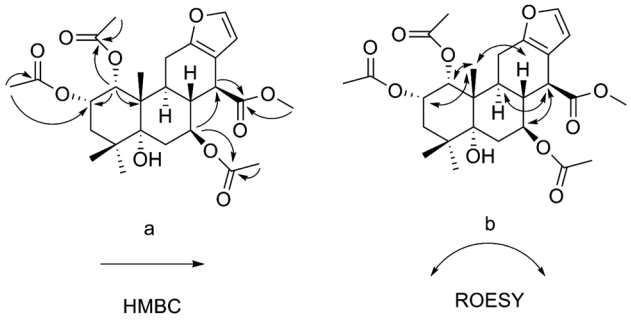

Fig. 5. Key HMBC and ROESY of Compound **6**

assigned as **6**.

Compounds **1**–**6** were evaluated for their cytotoxicity against three human cancer cell lines (HepG-2, MCF-7 and MG-63) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. All compounds were inactive against all of the tested cell lines at a concentration of 100 μM.

Experimental

General Experimental Procedures Melting points (mp) were measured on an X-4 digital display micro-melting apparatus, uncorrected. Optical rotations were determined with a JASCO P-1020 polarimeter. UV spectra were performed on a Shimadzu UV-2450 spectrophotometer. IR spectra were recorded in KBr-disc on a Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were acquired on a Bruker AV-500 NMR instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$. HR-ESI-MS was carried out on an Agilent UPLC-Q-TOF (6520B). Column chromatography (CC) was done using silica gel (Qingdao marine Chemical Co., Ltd., China), ODS (40–63 μm, FuJi, Japan), or Sephadex LH-20 (Pharmacia, Sweden). Preparative HPLC was carried out using a Shimadzu LC-6A instrument with a SPD-10A detector using a shim-pack RP-C18 column (20×200 mm). Analytical HPLC was measured on an Agilent 1200 Series instrument with a DAD detector using a shim-pack VP–ODS column (250×4.6 mm).

Plant Material The seeds of *Caesalpinia bonduc* (Linn.) Roxb. (Fabaceae) were purchased from Chengdu City, Sichuan province of China in March 2012, and were authenticated by Professor Min-Jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. CC201203) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The powdered air-dried seed kernels of *C. bonduc* (2.8 kg) were extracted with 95% EtOH (3×4 h). The EtOH extract was concentrated under reduced pressure. The crude extract (790 g) was suspended in water and successively partitioned with petroleum ether, CH_2Cl_2. The petroleum ether fraction, the CH_2Cl_2 fraction yielded 360 g, 142 g after removal of the solvent, respectively. Fractionation of the CH_2Cl_2 extract was performed on a silica gel column using a gradient of petroleum ether–acetone (50 : 1, 25 : 1, 10 : 1, 3 : 1) to yield four fractions 1–4 by TLC analysis. Fraction C2 (7 g) was run on an octadecyl silica (ODS) column using a step gradient of MeOH–H_2O (60 : 40 to 100 : 0), to afford four subfractions (C2.1–2.4). Fraction C2.4 was purified by preparative HPLC using the mobile phase MeOH–H_2O (70 : 30) to yield **1** (20 mg). Fraction C2.2 was chromatographed over a Sephadex LH-20 column, eluted with MeOH to give **4/5** (9 mg). Fraction C2.1 was chromatographed over an ODS column with a continuous gradient of MeOH–H_2O (50 : 50 to 100 : 0) to afford **6** (1.5 mg). Fraction C2.3 was eluted with MeOH–H_2O (70 : 30) on an ODS column and further purified by preparative HPLC using the mobile phase MeOH–H_2O (70 : 30) to yield **2** (3 mg).

Caesall H (**1**): colorless crystals; mp 204–206°C; $[\alpha]_D^{25}$ +79.6° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (logε) 211 (5.65), 250 (5.28), 280 (4.54), 291 (4.55) nm; IR (KBr) ν_{max} 3454, 1729, 1639, 1402, 1244 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 395.1830 $[M+Na]^+$ (Calcd for $C_{22}H_{28}NaO_5$: 395.1829).

Caesall I (**2**): White powder; $[\alpha]_D^{25}$ +114.6° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (logε) 210 (5.35), 250(5.02), 280 (4.40), 290(4.37) nm; IR (KBr) ν_{max} 3444, 2320, 1638, 1400, 1241, 1045 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 371.1850 $[M+H]^+$ (Calcd for $C_{22}H_{27}O_5$: 371.1853).

Caesall J (**3**): White powder; $[\alpha]_D^{25}$ −23.3° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε) 202 (3.03), 216 (sh) (2.70) nm; CD (MeOH) 215 ($\Delta\varepsilon$+9.0), 233 ($\Delta\varepsilon$+4.7), 260 ($\Delta\varepsilon$+12.4) nm; IR (KBr) ν_{max} 3455, 2355, 1749, 1637, 1402, 1256 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 543.2204 $[M+Na]^+$ (Calcd for $C_{27}H_{36}NaO_{10}$: 543.2201).

Caesall K/L (**4/5**, 1 : 1 diastereomixture): White powder; $[\alpha]_D^{25}$ −4.9° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (logε) 205 (4.21), 282 (2.30) nm; IR (KBr) ν_{max} 3453, 1743, 1640, 1399, 1385, 1250, 1036 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 455.2043 $[M+Na]^+$ (Calcd for $C_{24}H_{32}NaO_7$: 455.2040).

Caesall M (**6**): White powder; $[\alpha]_D^{25}$ −17.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 215 (3.93) nm; IR (KBr) ν_{max} 3578, 3467, 2955, 1742, 1367, 1241 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 543.2198 $[M+Na]^+$ (Calcd for $C_{27}H_{36}NaO_{10}$: 543.2201).

X-Ray Crystallographic Analysis Colorless crystals of **1** were obtained from CH_2Cl_2–MeOH. Crystal data were obtained on a Bruker Smart-1000 CCD with a graphite monochromator with CuKα radiation (λ=1.54184 Å) at 290(2) K. The structure was solved by direct methods using the SHELXS-97[15] and expanded using difference Fourier techniques, refined with the SHELXL-97.[16] Crystal data of **1**: $C_{22}H_{28}O_5$ (M=372.44); monoclinic crystal (0.37×0.36×0.3 mm^3); space group $P2_1$; unit cell dimensions a=10.7688(2) Å, b=7.7077(10) Å, c=11.7281(3) Å, β=99.691(2)°, V=959.57(3) Å^3; Z=2; D_{calcd}=1.289 mg/m^3; μ=0.733 mm^{-1}; 7749 reflections measured (7.64≤2Θ≤139.32); 3385 unique (R_{int}=0.0200) which were used in all calculations; the final

refinement gave R_1=0.0332 (>2sigma(I)) and wR_2=0.0950 (all data); flack parameter=0.04(16). Crystallographic data for compound **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 981573). Copies of the data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK. [fax: (+44)1223–336–033 or e-mail: deposit@ccdc.cam.ac.uk].

Bioassays The cytotoxicity of compounds **1**–**6** was assessed *via* the MTT method using the HepG-2, MG-63 and MCF-7 cancer cell lines. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and cultured at a density of 5000 cell/mL in a 96-well microtiter plate. Five different concentrations of each compound in dimethyl sulfoxide (DMSO) were subsequently added to the wells. Each concentration was tested in triplicate. After incubation under 5% CO_2 at 37°C for 48 h, 20 μL of MTT (4 mg/mL) was added to each well, and the cells were incubated for another 4 h. Then, the liquid in each well was removed, and DMSO (150 μL) was added. The absorbance (OD values) at 570 nm with a 630 nm reference was measured on a Universal Microplate Reader.

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