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Abietane Diterpenoids from *Coleus xanthanthus*

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Eight new abietane diterpenoids, coleon U 11-acetate (**1**), 16-acetoxycoleon U 11-acetate (**2**), and xanthanthusins F−K (**3**−**8**), together with five known analogues, coleon U (**9**), 16-*O*-acetylcoleon C (**10**), coleon U-quinone (**11**), 8α,9α-epoxycoleon U-quinone (**12**), and xanthanthusin E (**13**), were isolated from the aerial parts of *Coleus xanthanthus*. The structures of **1**−**8** were elucidated on the basis of spectral methods. Compounds **1**, **5**, and **11**−**13** were evaluated for their cytotoxicity against K562 human leukemia cells.

Coleus xanthanthus C. Y. Wu et Y. C. Huang (Labiatae), an endemic plant in the People's Republic of China, is distributed in the Xishuangbanna region of Yunnan Province up to an altitude of 1450 m.[1] This plant has use as a folk medicine for the treatment of rheumatic arthritis, colds, coughs, injuries from falls, neurasthenia, scabies, snakebites, and tuberculosis. The chemical constituents of *C. xanthanthus* have been investigated previously, and seven abietane diterpenoids (xanthanthusin A−E)[2−4] were characterized. Interestingly, certain abietane diterpenoids have been reported to exhibit potent antitumor activity.[5,6] To obtain potentially bioactive diterpenoids from *C. xanthanthus*, we have reinvestigated this plant collected from the southern part of Yunnan Province. As a result, 13 abietane diterpenoids (including eight new compounds) were isolated and identified. Some of these (**1**, **5**, **11**, **12**, and **13**) were bioassayed for their cytotoxic activity, and compounds **1**, **5**, **11**, and **12** showed significant inhibitory activity against K562 human leukemia cells. Herein, we present the structure elucidation of compounds **1**−**8** and the cytotoxic evaluation of compounds **1**, **5**, and **11**−**13**.

Results and Discussion

A 70% acetone extract prepared from the aerial parts of *C. xanthanthus* was partitioned between petroleum ether and H_2O, EtOAc and H_2O, and *n*-BuOH and H_2O, respectively. The petroleum ether layer was chromatographed repeatedly by column chromatography on silica gel and RP-18 and then subjected to preparative thin-layer chromatography, yielding 13 compounds (**1**−**13**).

Coleon U 11-acetate (**1**), obtained as yellow cubic crystals, was found to possess a molecular formula of $C_{22}H_{28}O_6$, as determined by HRFABMS (*m*/*z* 387.1887 [M − H]$^-$), combined with the analysis of its NMR data. The IR spectrum indicated hydroxyl (3416 cm^{-1}), ester (1747 cm^{-1}), conjugated ketone (1649 cm^{-1}), and aromatic ring (1620, 1599, and 1502 cm^{-1}) absorptions. The UV absorptions at 367.5, 283.5, and 258 nm supported the presence of a conjugated ketone and an aromatic ring. Its ^{1}H NMR spectrum exhibited signals for three tertiary methyls, one isopropyl, three methylenes, one acetoxyl, and two hydroxyl groups. From the ^{13}C NMR spectrum, besides the signals

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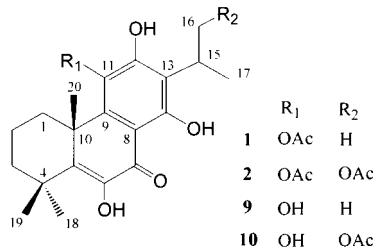
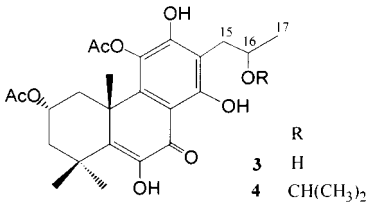
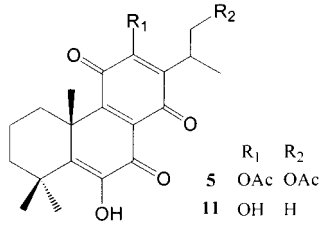


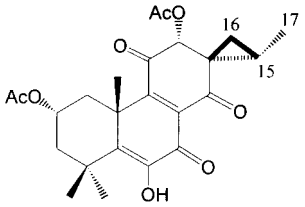
	R_1	R_2
1	OAc	H
2	OAc	OAc
9	OH	H
10	OH	OAc



	R
3	H
4	$CH(CH_3)_2$



	R_1	R_2
5	OAc	OAc
11	OH	H



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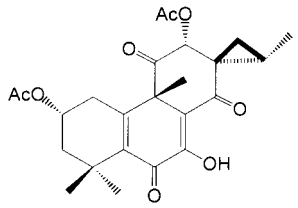

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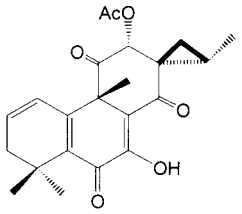

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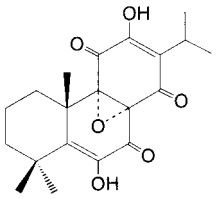

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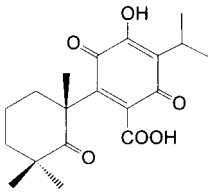

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from proton-bearing and quaternary carbons, a conjugated ketone and several aromatic ring carbons were also observed. Considering the diterpenoids previously isolated from this plant,[2] **1** could be assigned as an abietane diterpenoid with the basic skeleton abieta-5,8,11,13-tetraen-7-one and with the signals at δ 143.3 (s) and 142.0 (s) attributed to C-5 and C-6, respectively. The absence of vinylic proton signals implied that four substituents are present at C-6, C-11, C-12, and C-14. The conclusion was verified by the HMBC experiment with the major correlations shown in Figure 1. One hydroxyl group was placed at C-6 due to the correlations observed between the signal at δ_H 6.94 (1H, s, HO-6) and the signals at δ_C 182.3 (s, C-7), 143.3 (s, C-5), and 142.0 (s, C-6) in the HMBC spectrum. Comparison of the ^{13}C NMR spectral data of **1** with those of coleon U (**9**)[7] revealed that the signals of C-8, C-9, C-12, C-13, and C-14 of **1** were shifted downfield from those of coleon U, while the signal of C-11 of **1** was shifted upfield from that of coleon U, suggesting that an acetoxyl group was placed at the C-11 position in **1**. Accordingly, the other two hydroxyl groups were at C-12 and C-14. Thus, compound **1** was elucidated as 11-acetoxy-6,12,14-trihydroxyabieta-5,8,11,13-tetraen-7-one and was assigned the trivial name coleon U 11-acetate.

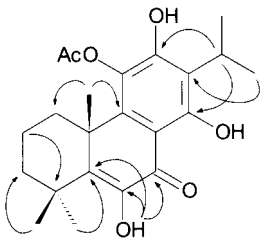

Figure 1. Selected HMBC correlations of **1**.

16-Acetoxycoleon U 11-acetate (**2**) differed from **1** only in possessing one more acetoxyl group. Comparison of their ^{1}H and ^{13}C NMR spectra indicated that the additional acetoxyl group was at the C-16 position in **2**. Correlations of the signals at δ_H 4.41 and 4.30 with the signals at δ_C 169.2 (s, AcO), 115.9 (s, C-13), 29.1 (d, C-15), and 14.9 (q, C-17) in the HMBC spectrum of **2** confirmed that the acetoxyl group is located at the C-16 position. Therefore, compound **2** was characterized as 11,16-diacetoxy-6,12,14-trihydroxyabieta-5,8,11,13-tetraen-7-one and was assigned the trivial name 16-acetoxycoleon U 11-acetate.

The ^{1}H NMR spectrum of xanthanthusin F (**3**) exhibited the signals of three tertiary methyls, two acetoxyl groups, and two hydroxyl groups, and from its ^{13}C NMR spectrum, a conjugated ketone system and aromatic ring were also observed, which indicated that **3** is an abietane diterpenoid with a structure similar to **1** and **2**. Its ^{1}H−^{1}H COSY spectrum exhibited two fragments, $-CH_2CH(OR)CH_2-$ (C-1 to C-3) and $-CH_2CH(OR)CH_3$ (C-15 to C-17), which suggested that two oxygenated substituents were placed at the C-2 and C-16 positions, respectively, and the side chain of **3** is not an isopropyl but rather an *n*-propyl group (CH_3-17 shifted to C-16 from C-15). From its HMBC experiment, the correlation of H-2 with AcO (δ 170.3, s) indicated an acetoxy to be placed at C-2 in **3**. The orientation of AcO-2 was determined from its ROESY spectrum,

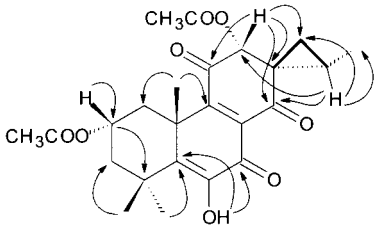

Figure 2. Selected HMBC correlations of **6**.

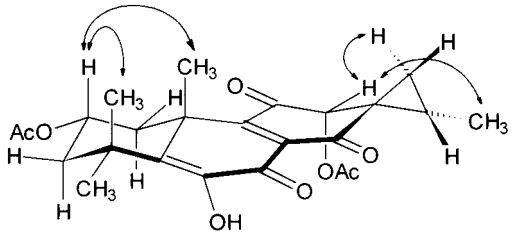

Figure 3. Key ROESY correlations of **6**.

and the correlations of H-2 with Me-19 and Me-20 confirmed that the C-2 acetoxy substituent is in an α-orientation. Thus, xanthanthusin F (**3**) was elucidated as 17-(15→16)-*abeo*-2α,11-diacetoxy-6,12,14,16-tetrahydroxyabieta-5,8,11,13-tetraen-7-one.

Comparison of the NMR spectra of xanthanthusin G (**4**) with those of **3** suggested that they are similar except that **4** possesses one more $-OCH(CH_3)_2$ group, which was determined to be at C-16 by the HMBC experiment. Accordingly, xanthanthusin G (**4**) was determined as 17-(15→6)-*abeo*-2α,11-diacetoxy-16-*O*-isopropyl-6,12,14-trihydroxyabieta-5,8,11,13-tetraen-7-one.

The ^{1}H NMR spectrum of xanthanthusin H (**5**) exhibited signals for three tertiary methyls and one isopropyl group, where one methyl was an oxygenated methylene, with HO-6 resonating at δ 7.06 (1H, s). The ^{13}C NMR spectrum showed signals for three conjugated carbonyl groups (δ 177.1, 179.8, 183.5, of which one was assigned to C-7). Analysis of the spectral properties suggested **5** is an abietane quinone diterpenoid.[8] Comparison of the ^{13}C NMR spectral data of **5** with those of coleon U-quinone (**11**)[8] clearly showed the presence of an acetyl and an acetoxy, with one oxygenated methylene located on the isopropyl group in **5**. The placement of oxygenated substituents was determined by the HMBC experiment. The correlations of δ_H 7.06 (1H, s, HO) with C-7 and C-5 and of δ_H 4.72 (2H, m, H-16) with δ_C 170.9 (s, AcO), 31.0 (d, C-15), and 15.2 (q, C-17) suggested a hydroxyl group was at C-6 and an acetoxy was at the C-16 position, respectively. Consequently, a further acetoxy could be placed at the C-12 position. Therefore, **5** was assigned as 12,16-diacetoxy-6-hydroxyabieta-5,8,12-trien-7,11,14-trione.

Analysis of its NMR spectral data indicated that xanthanthusin I (**6**) is a 13,16-cycloabietane quinone diterpenoid.[9] It differed from the diterpenoids **1**−**5** in possessing a cyclopropyl group, which was confirmed by the HMBC spectrum from the correlations summarized in Figure 2. In addition, the correlations of H-12 (δ 5.07, 1H, s) with AcO (δ 169.9, s) and H-2 (δ 5.00, 1H, m) with the other AcO (δ 169.9, s) suggested that two acetoxyl groups should be placed at C-12 and C-2, respectively. The stereochemistry of **6** was determined by the ROESY experiment. Correlations of H-2 with Me-19 and Me-20 and H-12 with Me-17 and H-16a were observed (Figure 3), suggesting that H-2 and H-12 have a β-orientation and that the chiral carbons C-13 and C-15 are in the *S* configuration by comparing the NMR spectral property of **6** to that of barbatusin.[10,11] Thus, xanthanthusin I (**6**) was elucidated as (13*S*,15*S*)-2α,12α-diacetoxy-6-hydroxy-13,16-cycloabieta-5,8-dien-7,11,14-trione.

Comparison of the ^{1}H and ^{13}C NMR spectral data of xanthanthusin J (**7**) with **6** suggested they are isomers. The structural elucidation of **7** was established mainly on the basis of its HMBC spectrum. The proton signals of Me-20 correlated with the signals of C-8 and C-11 but not with those of C-1 and C-5, indicating that Me-20 should be located at C-9. The HMBC experimental results also showed that there were two double bonds at C-5 to C-10, and C-7 to C-8. Moreover, C-6, C-11, and C-14 were assigned as three carbonyl carbons, and C-7 bore a hydroxyl group, while two acetoxyl groups were attached to C-2 and C-12. The ROESY spectrum showed that H-2 correlated with Me-19 and H-12 with Me-20 and H-16a, thereby suggesting that Me-20 is β-oriented. Accordingly, xanthanthusin J (**7**) was identified as (9*S*,13*S*,15*S*)-20-(10→9)-*abeo*-2α,12α-diacetoxy-7-hydroxy-13,16-cycloabieta-5(10),7-dien-6,11,14-trione.

Xanthanthusin K (**8**) differed from **7** in the lack of an acetoxyl group as well as the presence of one more double bond. The ^{1}H−^{1}H COSY spectrum of **8** revealed that the double bond was located between C-1 and C-2. Thus, xanthanthusin K (**8**) was determined to be (9*S*,13*S*,15*S*)-20-(10→9)-*abeo*-12α-acetoxy-7-hydroxy-13,16-cycloabieta-1,5(10),7-trien-6,11,14-trione.

The five known compounds were identified as coleon U (**9**),[7] 16-*O*-acetylcoleon C (**10**),[12] coleon U-quinone (**11**),[8] 8α,9α-epoxycoleon U-quinone (**12**),[8] and xanthanthusin E (**13**),[4] respectively, by comparing their spectral data with those reported in the literature.

Compounds **1**, **5**, **11**, **12**, and **13** were tested for their ability to inhibit K562 human leukemia cells, with mitoxanthrone used as the positive control substance.[13] The IC_{50} values for **1**, **5**, **11**−**13**, and mitoxanthrone were 2.2, 12.9, 3.0, 13.9, 34.3, and 2.0 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on an XRC-1 micromelting point apparatus and are uncorrected. Optical rotations were taken on a SEPA-300 polarimeter. UV spectra were obtained on a UV 210A spectrometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were run on Bruker AM-400 and DRX-500 instruments with TMS as internal standard, respectively. MS were recorded on a VG Auto Spec-3000 spectrometer. Si gel (200−300 mesh) for column chromatography and GF_{254} for TLC and preparative TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. RP-18 (40−63 μm) was obtained from Merck, Darmstadt, Germany.

Plant Material. The aerial parts of *Coleus xanthanthus* C. Y. Wu et Y. C. Huang were collected in the Xishuangbanna region of Yunnan Province, People's Republic of China, in September 1999 and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen (KIB 99-9-105 Lin) has been deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered aerial parts of the title plant (8.6 kg) were extracted with 70% Me_2CO and filtered. The filtrate was concentrated and extracted in turn with petroleum ether and EtOAc. The petroleum ether extract (130 g) was subjected to column chromatography on Si gel (1 kg) eluting with a petroleum ether−Me_2CO (1:0 → 0:1) gradient system to furnish fractions I−V. The fractions were combined after monitoring by TLC. Fraction I was purified by repeated column chromatography over Si gel (petroleum ether−Me_2CO, 30:1) to afford **9** (60 mg) and a mixture. The mixture was further purified by column chro-

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**8** (100.4 MHz, $CDCl_3$)

carbon	1	2	3	4	5	6	7	8
1	32.3 t	32.2 t	38.0 t	38.0 t	30.8 t	35.1 t	33.9 t	124.2 d
2	18.6 t	18.6 t	68.5 d	68.5 d	17.8 t	66.9 d	66.9 d	135.3 d
3	36.4 t	36.5 t	41.6 t	41.7 t	36.4 t	41.0 t	45.4 t	40.7 t
4	36.7 s	36.8 s	36.0 s	36.0 s	36.4 s	34.8 s	36.5 s	33.3 s
5	143.3 s	143.6 s	140.6 s	141.4 s	143.9 s	139.3 s	139.9 s	137.2 s
6	142.0 s	142.0 s	141.5 s	140.4 s	146.4 s	145.8 s	188.1 s	179.5 s
7	182.3 s	182.4 s	182.2 s	182.3 s	177.1 s	176.8 s	153.3 s	155.7 s
8	106.0 s	105.9 s	105.3 s	105.1 s	126.9 s	130.5 s	120.3 s	117.4 s
9	143.8 s	144.9 s	143.2 s	143.1 s	158.1 s	161.5 s	52.1 s	51.1 s
10	41.1 s	41.3 s	40.9 s	40.8 s	42.0 s	40.7 s	152.0 s	148.9 s
11	129.4 s	129.9 s	130.5 s	130.5 s	179.8 s	194.7 s	198.9 s	199.1 s
12	152.8 s	153.8 s	155.9 s	156.4 s	150.2 s	77.3 d	78.4 d	77.6 d
13	120.4 s	115.9 s	112.2 s	112.4 s	135.8 s	34.3 s	35.3 s	35.1 s
14	160.1 s	160.0 s	160.0 s	159.9 s	183.5 s	190.4 s	199.7 s	199.7 s
15	24.5 d	29.1 d	30.8 t	30.8 t	31.0 d	22.3 d	21.2 d	21.0 d
16	20.0 q	68.5 q	70.3 d	74.2 d	66.3 t	25.6 t	29.4 t	30.4 t
17	20.0 q	14.9 q	22.7 q	19.0 q	15.2 q	12.6 q	13.8 q	14.1 q
18	27.7 q	27.8 q	28.0 q	28.0 q	29.4 q	27.5 q	28.8 q	27.0 q
19	27.0 q	27.0 q	27.2 q	27.2 q	27.1 q	26.2 q	27.4 q	24.9 q
20	30.4 q	30.3 q	29.4 q	29.1 q	27.6 q	29.1 q	29.9 q	28.0 q
1′				70.1 d				
2′				23.3 q				
3′				21.1 q				
AcO	169.7 s	170.9 s	168.7 s	168.4 s	167.8 s	169.9 s	170.6 s	169.8 s
	21.3 q	21.2 q	20.9 q	20.9 q	20.2 q	20.1 q	21.3 q	20.2 q
AcO		169.2 s	170.3 s	170.2 s	170.9 s	169.9 s	169.9 s	
		20.9 q	21.3 q	21.3 q	20.8 q	21.0 q	20.2 q	

matography on RP-18 (MeOH−H_2O, 7:3) and led to the separation of **11** (50 mg) and **12** (40 mg). Fraction II was subjected to column chromatography over Si gel (petroleum ether−Me_2CO, 20:1) to give **1** (2.7 g), and the residue was purified by preparative TLC on Si gel (hexane−Me_2CO, 5:1, R_f 0.47) to yield **10** (18 mg). Fraction III was purified by repeated column chromatography over Si gel (petroleum ether−Me_2CO) and RP-18 (MeOH−H_2O) to afford **2** (20 mg), **3** (11 mg), **4** (4 mg), and **5** (30 mg), respectively. Fraction IV was separated by column chromatography over Si gel (petroleum ether−Me_2CO) and RP-18 (MeOH−H_2O), yielding **6** (270 mg) and **7** (37 mg), while **8** (17 mg) was purified by preparative TLC on Si gel (hexane−Me_2CO, 5:1, R_f 0.32). Fraction IV was subjected to column chromatography over Si gel (CH_2Cl_2−Me_2O, 20:1) and RP-18 (MeOH−H_2O, 6:4) to yield **13** (20 mg).

Coleon U 11-acetate (1): yellow cubic crystals (hexane−Me_2CO); mp 190.5−192 °C; $[\alpha]^{13.5}_D$ +20.5° (*c* 0.88, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 367.5 (3.89), 283.5 (3.96), 258 (3.74) nm; IR (KBr) ν_{max} 3416, 1747, 1649, 1620, 1599, 1541 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.46 (1H, m, H-1β), 1.88 (1H, m, H-1α), 1.79 (1H, m, H-2β), 1.41 (1H, m, H-2α), 1.91 (1H, m, H-3β), 1.42 (1H, m, H-3α), 6.94 (1H, s, HO-6), 13.19 (1H, s, HO-14), 3.45 (1H, m, H-15), 1.34 (6H, d, J = 6.8 Hz, Me-16, 17), 1.48 (3H, s, Me-18), 1.41 (3H, s, Me-19), 1.56 (3H, s, Me-20), 2.36 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m/z* 388 $[M]^+$ (44), 373 $[M - Me]^+$ (13), 346 $[MH - Me - CO]^+$ (55), 331 $[MH - 2 \times Me\text{-}CO]^+$ (100), 303 (17), 276 (63), 233 (16); negative-ion HRFABMS *m/z* 387.1857 (calcd for $C_{22}H_{27}O_6$, 387.1866).

16-Acetoxycoleon U 11-acetate (2): yellow amorphous powder; $[\alpha]^{25.9}_D$ +32.3° (*c* 0.87, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 368.5 (3.65), 284 (3.56), 258.5 (3.89) nm; IR (KBr) ν_{max} 3404, 1776, 1740, 1713, 1649, 1623, 1598, 1564 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.42 (1H, m, H-1β), 1.88 (1H, m, H-1α), 1.80 (1H, m, H-2β), 1.59 (1H, m, H-2α), 1.91 (1H, m, H-3β), 1.41 (1H, m, H-3α), 3.77 (1H, m, H-15), 4.41 (1H, dd, J = 6.0, 10.8 Hz, H-16a), 4.30 (1H, dd, J = 5.6, 10.8 Hz, H-16b), 1.38 (3H, d, J = 6.0 Hz, Me-17), 1.41 (3H, s, Me-18), 1.43 (3H, s, Me-19), 1.53 (3H, s, Me-20), 2.05 (3H, s, AcO), 2.34 (3H, s, AcO), 6.91 (1H, s, HO), 13.27 (1H, s, HO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m/z* 446 $[M]^+$ (63), 404 $[MH - Me - CO]^+$ (71), 386 $[M - CH_3COOH]^+$ (14), 344 $[MH - Me - CO - CH_3COOH]^+$ (66), 329 $[MH - 2 \times Me - CO - CH_3\text{-}COOH]^+$ (100), 275 (75), 233 (20); HREIMS *m/z* 446.1920 (calcd for $C_{24}H_{30}O_8$, 446.1941).

Xanthanthusin F (3): yellow amorphous powder; $[\alpha]^{25.8}_D$ +39.4° (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 379 (3.68), 320 (3.66), 279 (4.12), 268 (4.45) nm; IR (KBr) ν_{max} 3445, 1735, 1623, 1603, 1456 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.63 (1H, dd, J = 5.2, 14.8 Hz, H-1β), 2.19 (1H, dd, J = 8.4, 14.8 Hz, H-1α), 5.47 (1H, m, H-2β), 2.10 (1H, dd, J = 4.4, 13.6 Hz, H-3β), 1.89 (1H, dd, J = 7.2, 13.6 Hz, H-3α), 2.98 (1H, dd, J = 1.8, 14.8 Hz, H-15a), 2.87 (1H, dd, J = 6.8, 14.8 Hz, H-15b), 4.29 (1H, m, H-16), 1.24 (3H, d, J = 6.0 Hz, Me-17), 1.46 (6H, s, Me-18, Me-19), 1.60 (3H, s, Me-20), 6.92 (1H, s, HO), 13.00 (1H, s, HO), 2.06 (3H, s, AcO), 2.36 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m/z* 462 $[M]^+$ (25), 420 $[MH - Me - CO]^+$ (54), 402 $[M - CH_3COOH]^+$ (16), 342 $[M - 2 \times CH_3COOH]^+$ (61), 327 $[M - Me - 2 \times CH_3COOH]^+$ (100), 273 (24), 231 (12); HREIMS *m/z* 462.1930 (calcd for $C_{24}H_{30}O_9$, 462.1890).

Xanthanthusin G (4): yellow amorphous powder; $[\alpha]^{25.7}_D$ +35.0° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 389 (3.88), 327.5 (3.86), 281 (4.19), 268 (4.38) nm; IR (KBr) ν_{max} 3464, 1730, 1643, 1603, 1556 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.74 (1H, dd, J = 5.1, 15.0 Hz, H-1β), 2.17 (1H, dd, J = 8.7, 15.0 Hz, H-1α), 5.08 (1H, m, H-2β), 2.07 (1H, dd, J = 4.8, 14.0 Hz, H-3β), 1.90 (1H, dd, J = 7.5, 14.0 Hz, H-3α), 2.91 (2H, m, H_2-15), 3.94 (1H, m, H-16), 1.18 (3H, d, J = 5.6 Hz, Me-17), 1.46 (6H, s, Me-18, 19), 1.59 (3H, s, Me-20), 3.76 (1H, m, H-1′), 1.21 (3H, d, J = 6.0 Hz, Me-2′), 1.10 (3H, d, J = 6.0 Hz, Me-3′), 6.92 (1H, s, HO), 12.98 (1H, s, HO), 2.06 (3H, s, AcO), 2.32 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m/z* 504 $[M]^+$ (12), 462 $[MH - Me - CO]^+$ (59), 402 $[MH - Me - CO - CH_3COOH]^+$ (10), 342 $[MH - Me - CO - 2 \times CH_3\text{-}COOH]^+$ (52), 327 $[MH - 2 \times Me - CO - 2 \times CH_3COOH]^+$ (60), 273 (16), 231 (12), 43 (100); HREIMS *m/z* 504.2371 (calcd for $C_{27}H_{36}O_9$, 504.2359).

Xanthanthusin H (5): yellow amorphous powder; $[\alpha]^{25.7}_D$ −135.0° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 266 (4.11), 239 (3.04) nm; IR (KBr) ν_{max} 3386, 1780, 1742, 1681, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.52 (1H, m, H-1β), 1.73 (1H, m, H-1α), 1.87 (1H, m, H-2β), 1.71 (1H, m, H-2α), 2.08 (1H, m, H-3β), 1.61 (1H, m, H-3α), 3.40 (1H, m, H-15), 4.27 (2H, m, H_2-16), 1.26 (3H, d, J = 6.8 Hz, Me-17), 1.43 (6H, s, Me-18, 19), 1.75 (3H, s, Me-20), 7.06 (1H, s, HO), 2.35 (3H, s, AcO), 1.98 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m/z* 446 $[MH_2]^+$ (7), 404 (13), 344 (15), 329 (52), 275 (27), 233 (31), 43 (100); HREIMS *m/z* 446.1942 (calcd for $C_{24}H_{30}O_8$, 446.1941).

Xanthanthusin I (6): yellow amorphous powder; $[\alpha]^{24.8}_D$ −49.5° (*c* 0.46, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 369.5 (3.01), 244 (4.58) nm; IR (KBr) ν_{max} 3336, 1755, 1726, 1700, 1650, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.97 (1H, d, J = 6.2 Hz, H-1β), 1.85 (1H, hidden, H-1α), 5.00 (1H, m, H-2β), 2.06 (1H, hidden, H-3β), 1.85 (1H, hidden, H-3α), 5.07 (1H, s, H-12β), 1.93 (1H, m, H-15), 1.28 (2H, m, H_2-16), 1.10 (3H, d, J = 6.4 Hz, Me-17), 1.39 (3H, s, Me-18), 1.36 (3H, s, Me-19), 1.75 (3H, s, Me-20), 8.83 (1H, s, HO), 2.06 (3H, s, AcO), 2.03 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m*/*z* 444 $[M]^+$ (7), 402 $[MH - Me - CO]^+$ (4), 384 $[M - CH_3COOH]^+$ (51), 342 $[MH - Me - CO - CH_3COOH]^+$ (34), 325 $[MH - 2 \times CH_3COOH]^+$ (65), 309 $[M - 2 \times CH_3COOH - Me]^+$ (92), 269 (70), 231 (17), 43 (100); HREIMS *m*/*z* 444.1778 (calcd for $C_{24}H_{28}O_8$, 444.1784).

Xanthanthusin J (7): yellow amorphous powder; $[\alpha]^{13.6}_D$ +261.3° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 352 (3.68), 332 (4.09), 270 (3.26) nm; IR (KBr) ν_{max} 3449, 1755, 1734, 1641, 1599, 1542 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.86 (1H, dd, J = 5.2, 18.7 Hz, H-1β), 2.42 (1H, dd, J = 9.6, 18.7 Hz, H-1α), 4.96 (1H, m, H-2β), 1.81 (1H, dd, J = 5.5, 12.4 Hz, H-3β), 1.59 (1H, dd, J = 10.4, 12.4 Hz, H-3α), 4.80 (1H, s, H-12β), 2.25 (1H, m, H-15), 1.50 (1H, dd, J = 4.0, 9.2 Hz, H-16a), 1.07(1H, dd, J = 4.0, 7.5 Hz, H-16b), 1.18 (3H, d, J = 6.5 Hz, Me-17), 1.39 (3H, s, Me-18), 1.31 (3H, s, Me-19), 1.69 (3H, s, Me-20), 11.58 (1H, s, HO), 2.04 (3H, s, AcO), 1.98 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m*/*z* 444 $[M]^+$ (2), 384 $[M - CH_3COOH]^+$ (100), 342 $[MH - Me - CO - CH_3COOH]^+$ (37), 324 $[M - 2 \times CH_3COOH]^+$ (92), 297 (78), 230 (73); HREIMS *m*/*z* 444.1752 (calcd for $C_{24}H_{28}O_8$, 444.1784).

Xanthanthusin K (8): yellow amorphous powder; $[\alpha]_D^{13.6}$ +261.9° (*c* 0.44, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 355 (3.86), 332 (4.12), 269.5 (3.69) nm; IR (KBr) ν_{max} 3440, 1734, 1642, 1599, 1542 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.22 (1H, d, J = 5.6 Hz, H-1), 6.31 (1H, m, H-2), 2.24 (1H, dd, J = 3.2, 15.2 Hz, H-3β), 2.14 (1H, dd, J = 4.8, 15.2 Hz, H-3α), 5.04 (1H, s, H-12β), 2.46 (1H, m, H-15), 1.52 (1H, dd, J = 4.0, 7.6 Hz, H-16a), 1.15(1H, dd, J = 4.0, 9.2 Hz, H-16b), 1.18 (3H, d, J = 6.0 Hz, Me-17), 1.33 (3H, s, Me-18), 1.19 (3H, s, Me-19), 1.74 (3H, s, Me-20), 12.37 (1H, s, HO), 2.00 (3H, s, AcO); ^{13}C NMR ($CDCl_3$, 100.4 MHz), see Table 1; EIMS *m*/*z* 384 $[M]^+$ (92), 342 $[MH - Me - CO]^+$ (50), 324 $[M - CH_3COOH]^+$ (91), 309 $[M - Me - CH_3COOH]^+$ (100), 297 (68), 269 (88), 230 (73); HREIMS *m*/*z* 384.1573 (calcd for $C_{22}H_{24}O_6$, 384.1573).

Cytotoxicity Against K562 Human Leukemia Cells. An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in 96-well plates. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically. K562 human leukemia cells at a log phase of their growth cycle (5×10^4 cell/mL) were added to each well (90 μL/well), then treated in four replicates at various concentrations of the drugs (1, 0.1, 0.01, 0.001, 0.0001 μg/mL), and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 10 μL of MTT solution (5 mg/mL) per well was added to each cultured medium and incubated for a further 4 h. Then, 10% SDS−5% isobutanol−0.012 moL/L HCl was added to each well (100 μL/well). After 12 h at room temperature, the optical density of each well was measured on an ELISA reader (Bioteck EL-340) at two wavelengths (570 and 630 nm). In these experiments, the negative reference agents were isochoric normal saline, 1% DMSO, or 0.1% DMSO, and mitoxantrone was used as the positive reference substance with concentrations of 100, 10, and 1 μg/mL.

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