Cytotoxic Pentacyclic Triterpenoids from *Combretum sundaicum* and *Lantana camara* as Inhibitors of Bcl-xL/BakBH3 Domain Peptide Interaction

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In an effort to discover potent inhibitors of the antiapoptotic protein Bcl-xL, a systematic in vitro evaluation was undertaken on extracts prepared from various parts of Vietnamese plants. The ethyl acetate extracts obtained from the leaves and flowers of *Combretum sundaicum* and the leaves of *Lantana camara* were selected for their interaction with the Bcl-xL/Bak association. Bioassay-guided purification of these species led to the isolation of 15 pentacyclic triterpenoids (**1**−**15**) possessing olean-12-en-28-oic acid and olean-12-en-29-oic acid aglycons, of which compounds **1**−**6** and **8**−**10** are new. Five compounds exhibited binding activity with K_i values between 5.3 and 17.8 μM. The cytotoxic activity of **1**−**15** was also evaluated on various cancer cell lines.

The antiapoptotic protein Bcl-xL, a member of the Bcl-2 family, has recently become an attractive molecular target for cancer treatment or prevention drug discovery.[1] This target was almost unknown before 1993, but the number of publications related to this protein has increased dramatically over the past few years, indicating a promising new laboratory approach. Indeed, agents that can down-regulate Bcl-2, Bcl-xL, and/or Mcl-1 expression or activity may be useful for cancer chemotherapy.[2] The discovery of potent inhibitors of Bcl-xL has resulted from NMR studies and parallel synthesis,[3] or has been based on computational structure-based modeling[4] or multiple high-throughput screening platforms.[5] On the other hand, recent studies have shown that natural compounds, such as gossypol,[6] chelerythrine,[7] incednine,[8] and meiogynine,[9] may constitute a promising source of new therapeutic agents that target Bcl-xL.

An affinity displacement assay utilizing fluorescence polarization, based on the binding of a fluorescein-labeled peptide (BH3-domain of Bak) to Bcl-xL, has recently been developed by Qian and co-workers.[5] Using this assay, a screen was conducted on 1895 ethyl acetate extracts prepared from various parts of 730 Vietnamese plants. The EtOAc extracts from the leaves and flowers of *Combretum sundaicum* Miquel (Combretaceae) and from the leaves of *Lantana camara* L. (Verbenaceae) were selected for their potency to modulate the interaction between Bcl-xL and Bak. This prompted us to perform a detailed bioassay-guided chemical investigation of these plants, leading to the isolation of a series of 15 pentacyclic triterpenoids (**1**−**15**), of which compounds **1**−**6** and **8**−**10** are new.

The family Combretaceae consists of 20 genera and about 600 species.[10] The genus *Combretum* includes about 250 species mainly distributed in South America, Africa, and, to a lesser extent, Southeast Asia from Vietnam to Malaysia. *C. sundaicum* is a climbing tree having opposite or subopposite leaves pubescent at the inner face; the flowers are small, white, regular, bisexual, and organized on a compound umbel. No phytochemical study has been carried out on *C. sundaicum,* but two papers have dealt with the constituents of the so-called "Malayan antiopium plant".[11,12] Many *Combretum* species have been studied in the past, leading to various types of compounds, such as the antimicrobial pentacyclic triterpenoids isolated from the African *C. imberbe*[13] and the well-known antifungal asiatic acid and arjunolic acid isolated from *C. nelsonii*.[14]

L. camara is a widespread plant generally considered very toxic to cattle,[15] probably due to the presence of lantadenes A (**11**) and B (**12**).[16] Different parts of this plant are used in traditional medicine for the treatment of various human ailments.[17] Several taxa have been described so far, but only the "Townsville Prickly Orange" taxon, characterized by the presence of yellow to orange flowers, has been reported to contain icterogenin (**14**).[18] A large amount of this compound was isolated from our plant material. An extensive literature exists on the chemistry and the biology of this species. It was recently demonstrated that lantadene A (**11**) induces efficient cell apoptosis (HL-60) by activating the caspase-3 pathway and through down- and up-regulation of Bcl-2 and Bax expression, respectively.[19] Lantadenes A (**11**) and B (**12**) and their methyl esters are also potent in vivo antitumor agents against squamous cell carcinogenesis.[20]

We report in this paper the isolation, characterization, and biological activities of 15 analogues of oleanolic acid (**1**−**15**), of which **11** and **13** were isolated as a 2:1 mixture.

Results and Discussion

Leaves and flowers of *C. sundaicum* and leaves of *L. camara* were extracted by ethyl acetate to give crude extracts. One hundred milligrams of each extract was filtered on polyamide to remove tannins, and 15 mg of the filtered extracts was fractionated on a semipreparative C_{18} column to give nine fractions according to a standardized method.[21] The bioassay used allowed us to identify one active fraction (F6, t_R from 42 to 50 min for *C. sundaicum*, and F5, t_R from 34 to 42 min for *L. camara*) in each extract, which was used for reference for further purification. The EtOAc extracts obtained from *C. sundaicum* leaves and flowers (22.1 and 5.1 g, respectively) were then subjected to several silica gel chromatographic steps. Flash chromatography on C_{18} was performed on the crude extract (6.4 g) of *L. camara* leaves. Comparative analytical HPLC of other fractions with the reference fraction F5 or F6 allowed us to target those containing the active compounds. Subsequent preparative HPLC purification resulted in the isolation of compounds **1**−**15**.

Ten pentacyclic olean-12-ene triterpenoids were isolated from *C. sundaicum* flowers (**2**, **6**), leaves (**7**−**10**), or both parts (**1**, **3**−**5**). The methyl ester compounds **2**, **6**, **8**, and **9** might be artifacts formed during the isolation process since a large proportion of methanol was used in the eluant. The IR spectrum of all compounds showed

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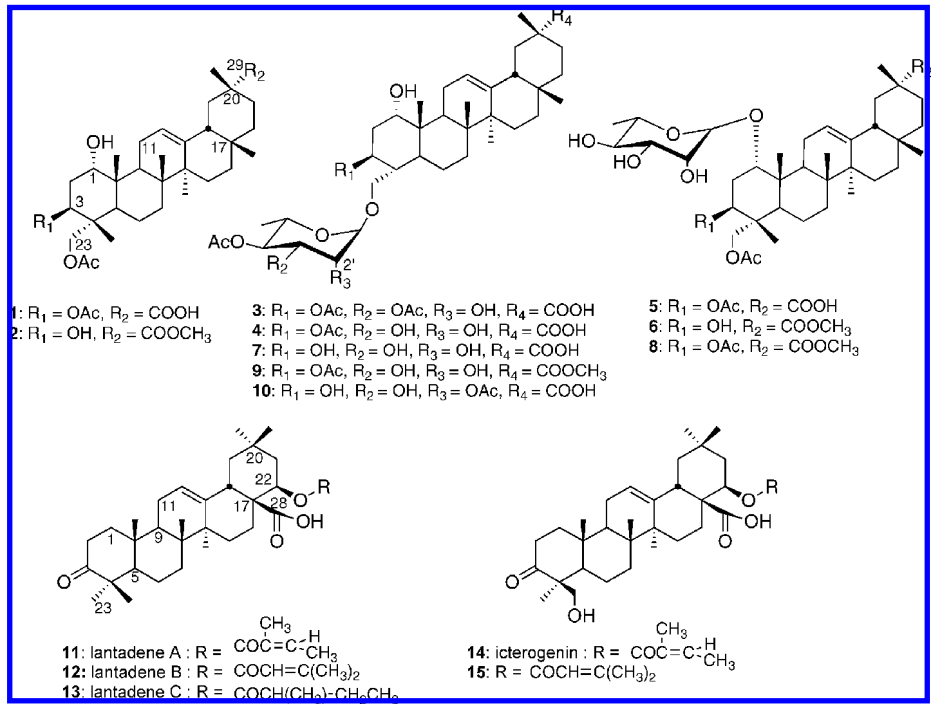
Chart 1



absorption bands for hydroxy groups at 2944−3456 cm^{-1} and carboxylic acids or carboxylic acid esters at 1716−1729 cm^{-1}. The NMR spectra were suggestive of triterpenoid-type compounds with carboxylic or methyl ester functions, a trisubstituted double bond, and an AB system. A thorough analysis of NMR data showed that the compounds were 23-hydroxyimberbic acid derivatives characterized by a 1α,3β-dihydroxyolean-12-ene-29-oic acid moiety.[22] Imberbic acid was isolated for the first time from *Combretum imberbe*,[23] and glycosidic derivatives possessing antimicrobial activity were isolated from the same species by Waigh and his group.[13] The presence of additional acetyl or glycosylated groups were systematically observed for all the compounds isolated. In the case of the glycosylated compounds, the sugar moiety was characterized as α-L-rhamnopyranose or mono- or diacetylated α-L-rhamnopyranose, the anomeric carbons of which normally appear around δ_C 101.5 ppm.[24] The L-isomer was confirmed by the measurement of the optical rotation, $[\alpha]^{25}_D$ −7.3 (*c* 0.1, MeOH), of the purified sugar obtained from acid hydrolysis and comparison with an authentic sample. The ^1H and ^{13}C NMR spectra of all compounds were very similar, with some variations in the region between δ_H 3.40 and 5.25 ppm (δ_C 60−102 ppm). The modifications appeared around the point of attachment of the acetyl and/or sugar units. Acetylation and glycosylation caused downfield shifts of the ^{13}C NMR resonances of the substituted carbons (about 3 and 10 ppm, respectively). Full ^1H and ^{13}C NMR assignments are given for compounds **1**−**6** and **8**−**10** in Tables 1 and 2.

Five other pentacyclic olean-12-ene triterpenoids, lantadenes A (**11**), B (**12**), and C (**13**) and icterogenin (**14**) and its regioisomer (**15**), were isolated from the leaves of *L. camara*. They were identified by comparison of their spectroscopic data (HR-MS, ^1H and ^{13}C NMR data) with those of analogues reported in the literature.[19,20] Their distribution in various *L. camara* taxa has been thoroughly discussed in the study of Hart et al.[18]

Compound **1** was isolated as a white, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 595.3583, which suggested a molecular formula of $C_{34}H_{52}O_7Na$ (calcd 595.3611). Analysis of the ^1H and ^{13}C NMR spectra suggested that compound **1** possesses a 23-hydroxyimberbic acid aglycon with two acetyl groups (Tables 1 and 2). The correlations observed in the COSY, HMBC, and NOESY spectra are depicted in Figure 1. The substitutions of the pentacyclic moiety by the two acetyl groups at positions 3 and 23 were deduced from the correlations from H-3 to C-5 (δ_C 40.9) and the carbonyl at δ_C 170.8, on one hand, and from H_2-23 to C-4 (δ_C 41.0) and the carbonyl at δ_C 184.5 on the other hand (Figure 1). In the NOESY spectrum, the correlations of H-3 and H-5 with H_2-23, H-9 with H-5 and H_3-27, H_{ax}-21 with H_3-28, H_{eq}-21 with H_3-30, and H_3-COO-3 with H_3-24 were in complete agreement with the relative configuration found for imberbic acid.[23] Thus, compound **1** was assigned as 23-acetoxy-3β-acetylimberbic acid.

Compound **2** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 567.3685, which suggested a molecular formula of $C_{33}H_{52}O_6Na$ (calcd 567.3662). The IR and ^1H and ^{13}C NMR spectroscopic data were almost identical to those of **1** (Tables 1 and 2). Upfield shifts of the resonances of the oxymethine CH-3 at δ_H 3.89, δ_C 67.5 (δ_H 5.22, δ_C 70.5 in compound **1**) suggested the presence of a hydroxy group at C-3 instead of an acetyl group in **1**. The presence of a methoxy group at δ_H 3.63 and δ_C 51.9, attached to C-29, was confirmed by the HMBC correlations from the methoxy protons to the carbonyl at δ_C 179.5 (Table 1). Other NMR correlations were similar to those of **1**. Compound **2** was proposed to be the 29-methyl ester of 23-acetoxyimberbic acid.

Compound **3** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 783.4280, which suggested a molecular formula of $C_{42}H_{64}O_{12}Na$ (calcd 783.4295). From this formula and the NMR data, we deduced that compound **3** possesses a 3′,4′-diacetylated rhamnose attached at C-23. An α-rhamnopyranose sugar unit was identified using ^1H and $^1H-^1H$ COSY spectra, starting from the anomeric proton at δ_H 4.62 (brs), on the basis of the correlations between H-1′ and H-2′ at δ_H 4.06 (brs), H-2′ and H-3′ at δ_H 5.11 (dd, J = 8.9, 1.3 Hz), H-3′ and H-4′ at δ_H 5.12 (t, J = 8.9 Hz), H-4′ and H-5′ at δ_H 3.73 (dq, J = 8.9, 5.4 Hz), and H-5′ and H_3-6′ at δ_H 1.19 (d, J = 5.4 Hz). The axial positions of H-3′, H-4′, and H-5′ were confirmed by the presence of large coupling constants within the axial−axial coupling range of 6−14 Hz and from cross-peaks between H-3′ and H-5′ in the NOESY spectrum. The substitution on the sugar moiety by two acetyl groups at positions 3′ and 4′ was deduced from correlations observed in the HMBC spectrum from H-3′ to

Table 1. ^{1}H NMR Spectroscopic Data (δ_H (J in Hz)) (500 MHz, $CDCl_3$) for Compounds **1**−**10**

position	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**9**	**10**
1	3.62, brs	3.61, t (2.5)	3.59, brs	3.62, brs	3.45, brs	3.44, brs	3.50, brs	3.60, brs	3.60, t (2.8)
2	1.87, 1.98, m	1.76, 1.99, m	1.77, 1.94, m	1.81, 1.96, m	1.90, 2.02, m	1.90, 2.01, m	nd, 1.95, m	1.81, 1.99, m	1.71, 1.96, m
3	5.22, dd (12, 4.4)	3.89, dd (12.2, 4.6)	5.29, dd (12.4, 4.3)	5.54, dd (12.2, 4.2)	5.28, dd (12.2, 4.9)	3.83, d (12.0)	5.28, dd (9.8, 7.2)	5.38, dd (12.4, 4.4)	4.09, dd (12.8, 4.3)
5	1.67, t (6.5)	1.47, m	1.94, m	1.82, m	1.74, m	1.42, m	1.62, m	1.80, m	1.60, d (9.2)
6	1.44, m	1.48, m	1.41, m	1.42, m	1.44, m	1.45, m	1.43, m	1.42, m	1.38, 1.47, m
7	1.28, 1.45, m	1.29, 1.48, m	1.29, 1.68, m	1.29, 1.51, m	1.28, 1.52, m	1.29, 1.43, m	1.28, 1.42, m	1.29, 1.51, m	1.28, 1.47, m
9	2.39, dd (10.8, 6.4)	2.30, dd (11.5, 6.4)	2.44, t (8.4)	2.33, t (8.8)	2.47, dd (11.7, 6.2)	2.34, dd (11.2, 5.6)	2.32, dd (12.1, 5.8)	2.39, dd (9.7, 7.9))	2.27, dd (11.1, 6.4)
11	1.87, 1.91, m	1.80, 1.94, m	1.84, m[a]	1.90, m[a]	1.59 1.91, m	1.60 1.92, m	1.63 1.94, m	1.89, m[a]	1.86, 1.93, m
12	5.21, t (3.4)	5.21, t (3.8)	5.19, t (3.3)	5.25, t (3.7)	5.18, t (3.1)	5.18, t (4.1)	5.18, t (3.3)	5.22, t (3.3)	5.24, t (3.3)
15	0.98, 1.74, m	0.98, 1.75, m	0.96, 1.77, m	0.95, 1.77, m	0.96, 1.73, m	0.98, 1.74, m	0.97, 1.73, m	0.96, 1.79, m	0.92, 1.78, m
16	1.98, m	1.24, 2.00, m	0.80, 2.01, m	nd, 2.03, m	0.81, 2.04, m	0.84, 1.98, m	0.83, 1.98, m	0.85, 1.97, m	nd, 2.01, m
18	1.89, m	1.97, m	1.95, m	1.98, m	1.96, m	1.97, m	1.96, m	1.97, m	1.99, m
19	1.97, 2.14, m	1.34, 2.11, m	1.27, 2.19, m	1.33, 1.97, m	1.26, 2.12, m	1.30, 2.10, m	1.30, 2.09, m	1.35, 2.10, m	1.37, 2.11, m
21	1.43, 1.87, m	1.40, 1.84, m	1.34, 1.94, m	1.98, 2.10, t (13.6)	1.87, nd, m	1.41, 1.81, m	1.40, 1.63, m	1.41, 1.83, m	1.37, 1.92, m
22	1.31, 1.43, m	1.30, 1.44, m	1.28, 1.39, m	1.30, 1.40, m	1.28, 1.44, m	1.31, 1.44, m	1.27, 1.43, m	1.30, 1.46, m	1.30, 1.40, m
23	3.71, d (11.5)	3.82, d (11.5)	2.83, d (9.8)	2.77, d (9.3)	3.67, d (11.7)	3.82, d (11.6)	3.69, d (12.1)	2.84, d (9.7)	3.29, d (9.2)
	3.90, d (11.5)	4.22, d (11.5)	3.45, d (9.8)	3.57, d (9.3)	3.77, d (11.7)	4.21, d (11.6)	3.85, d (12.1)	3.53, d (9.7)	3.51, d (9.2)
24	0.83. s	0.79. s	0.74. s	0.75. s	0.82. s	0.79. s	0.84. s	0.76. s	0.70. s
25	0.98, s	0.97, s	0.95, s	0.97, s	0.98, s	0.97, s	1.00, s	0.97, s	0.95, s
26	0.96, s	0.97, s	0.97, s	0.96, s	0.96, s	0.97, s	0.97, s	0.97, s	0.97, s
27	1.14, s	1.16, s	1.19, s	1.18, s	1.18, s	1.12, s	1.13, s	1.17, s	1.17, s
28	0.83, s	0.84, s	0.82, s	0.83, s	0.83, s	0.83, s	0.82, s	0.83, s	0.84, s
30	1.20, s	1.18, s	1.18, s	1.19, s	1.13, s	1.18, s	1.17, s	1.18, s	1.20, s
$OCOCH_3$-3	1.99, s		1.96, s	2.02, s	1.98, s		2.00, s	2.00, s	
$OCOCH_3$-23	2.05, s	2.09, s			2.02, s	2.09, s	2.05, s		
OCH_3-29		3.63, s				3.65, s	3.63, s	3.62, s	
1′			4.62, brs	4.66, brs	4.73, brs	4.79, brs	4.80, brs	4.63, brs	4.71, d (1.3)
2′			4.06, brs	3.90, brs	3.66, t (2.3)	3.78, brs	3.84, brs	4.00, brs	5.21, t (2.1)
3′			5.11, dd (8.9, 1.3)	3.95, dd (9.6, 1.9)	3.85, dd (9.3, 2.3)	3.64, dd (9.8, 1.9)	3.75, dd (9.1, 2.5)	3.88, dd (9.6, 2.7)	4.08, dd (9.9, 4.3)
4′			5.12, t, (8.9)	4.91, t, (9.6)	3.42, t, (9.7)	3.45, t, (9.8)	3.43, t, (9.1)	4.84, t, (9.6)	4.82, t, (9.9)
5′			3.73, dq (8.9, 5.4)	3.79, dq (9.6, 6.3)	4.03, dq (9.7, 6.1)	3.73, dq (9.8, 6.0)	4.00, dq (9.1, 5.8)	3.73, dq (9.6, 6.1)	3.80, dq (9.9, 6.2)
6′			1.19, d, (5.4)	1.15, d, (6.3)	1.76, d, (6.1)	1.30, d, (6.0)	1.27, d, (5.8)	1.17, d, (6.1)	1.18, d, (6.2)
$OCOCH_3$-2′									2.05, s
$OCOCH_3$-3′			2.01, s						
$OCOCH_3$-4′			1.96, s	2.01, s				2.08, s	2.17, s

[a] Designates poorly resolved chemical shifts and/or coupling constants.

Table 2. ^{13}C NMR Spectroscopic Data (δ_C) (125 MHz, $CDCl_3$) for Compounds **1**−**10**

position	1	2	3	4	5	6	8	9	10
1	71.8	72.1	71.4	71.7	82.6	82.7	81.3	71.9	72.4
2	30.7	33.5	30.3	30.0	28.8	31.0	28.8	30.6	33.6
3	70.5	67.5	71.2	70.9	70.2	67.9	70.7	70.5	66.1
4	41.0	42.4	41.4	41.4	41.1	42.2	41.6	41.4	40.9
5	40.9	41.3	39.8	40.2	41.1	41.7	41.2	40.2	40.6
6	18.1	18.3	18.4	18.0	18.0	18.3	18.1	18.0	18.2
7	31.9	32.0	32.1	31.6	31.7	32.1	32.1	31.7	31.8
8	39.7	39.8	39.8	39.8	39.7	39.9	39.9	42.3	39.8
9	38.1	38.3	38.3	38.3	37.4	37.9	37.8	38.2	38.4
10	41.0	41.0	40.5	40.8	40.9	41.5	41.0	40.9	40.9
11	23.4	23.4	23.3	23.3	23.6	23.7	23.9	23.4	23.4
12	122.7	122.4	123.0	123.1	121.7	122.1	122.0	122.8	123.0
13	144.3	144.6	143.7	143.9	145.7	145.1	145.1	144.2	144.2
14	42.2	42.9	42.8	42.3	42.5	42.4	42.2	42.4	42.3
15	26.2	26.3	26.4	26.3	26.0	26.2	26.1	26.4	26.4
16	27.1	26.2	27.0	26.8	27.2	27.0	27.0	27.1	26.8
17	32.6	32.6	32.7	32.6	32.6	32.6	32.6	32.6	32.6
18	46.1	46.2	46.6	46.1	46.0	46.2	46.2	46.2	46.1
19	40.4	40.7	41.1	41.8	41.2	40.5	40.3	40.7	41.4
20	42.7	42.8	42.7	42.8	42.3	43.0	42.9	42.9	42.8
21	29.1	29.2	28.5	28.1	28.2	29.3	29.3	29.2	28.4
22	36.0	36.1	35.9	35.9	36.1	36.0	36.0	36.1	35.9
23	65.8	68.0	69.3	69.2	66.2	67.3	65.7	69.9	70.7
24	13.1	11.9	12.8	12.8	13.5	12.1	13.2	13.1	12.0
25	16.9	17.0	16.6	17.2	16.1	16.4	16.4	16.9	16.8
26	17.1	17.1	17.7	16.6	17.0	17.2	17.2	17.2	17.2
27	26.1	26.2	26.3	26.3	27.0	26.6	26.6	26.1	26.2
28	28.4	28.4	28.5	28.4	28.4	28.3	28.3	28.4	28.4
29	184.5	179.5	186.3	186.7	182.3	179.9	179.7	179.5	185.8
30	19.4	19.6	18.9	18.9	19.3	19.5	19.4	19.6	19.1
$OCOCH_3$-3	170.8		171.7	172.5	170.3		170.9		
$OCOCH_3$-3	21.5		21.4	21.7	21.4		21.3		
$OCOCH_3$-23	171.4	171.8			171.7	171.9	171.3		
$OCOCH_3$-23	21.2	21.3			21.0	21.2	21.1		
OCH_3-29		51.9				52.1	52.1	52.0	
1′			101.4	100.0	101.7	102.1	102.1	100.2	97.4
2′			69.3	71.4	72.3	72.4	71.6	70.9	73.1
3′			71.3	70.2	71.7	71.7	72.5	70.4	68.7
4′			71.8	74.6	73.2	73.6	73.8	75.1	74.7
5′			66.9	67.1	70.4	69.2	69.3	66.3	66.6
6′			17.4	17.5	17.6	17.6	17.7	17.5	17.5
$COCH_3$-2′									171.7
$OCOCH_3$-2′									21.4
$OCOCH_3$-3′			170.5						
$OCOCH_3$-3′			21.2						
$OCOCH_3$-4′			170.3	171.7				171.7	171.8
$OCOCH_3$-4′			20.9	21.5				21.4	21.5

the carbonyl at δ_C 170.5 and from H-4′ to the carbonyl at δ_C 170.3 (Table 1). Finally, HMBC correlations from H-1′ to C-23 and H-3 to the carbonyl at δ_C 171.7 confirmed the location of the sugar unit and the acetyl group at C-23 and C-3, respectively. Compound **3** was thus established as 23-*O*-[α-L-(3′,4′-diacetylrhamnopyranosyl]-3*β*-acetylimberbic acid.

Compound **4** was purified as colorless crystals from a 1:1 MeOH−CH_2Cl_2 mixture at room temperature. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m*/*z* 741.4180, which suggested a sodiated molecular formula of $C_{40}H_{62}O_{11}Na$ (calcd 741.4190). The spectroscopic data of compound **4** were almost identical to those of compound **3** but with two methyl signals at δ_H 2.01 and 2.02 attributable to two acetoxy groups. In the ^1H NMR spectrum the H-3′ proton signal shifted upfield to δ_H 3.95 (dd, J = 9.6, 1.9 Hz), suggesting the absence of an acetate group attached at this position. The structure of compound **4**, confirmed by X-ray crystallographic analysis (Figure 2), was determined as 23-*O*-[α-L-(4′-acetylrhamnopyranosyl]-3*β*-acetylimberbic acid.

Compound **5** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m*/*z* 741.4219, which suggested a sodiated molecular formula of $C_{40}H_{62}O_{11}Na$ (calcd 741.4290), identical to compound **4**. The spectroscopic data

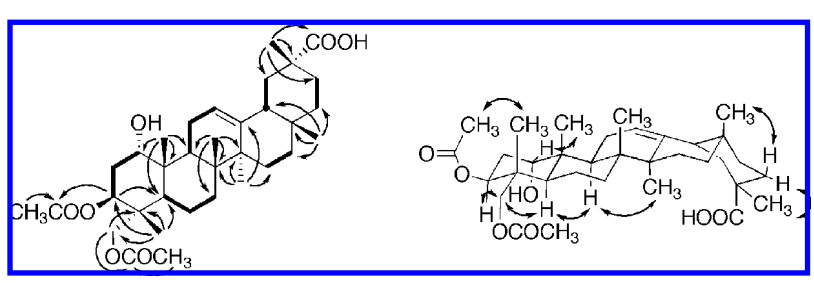


Figure 1. Key HMBC (arrows) and COSY (bold) (left) and NOESY (right) correlations for **1**.

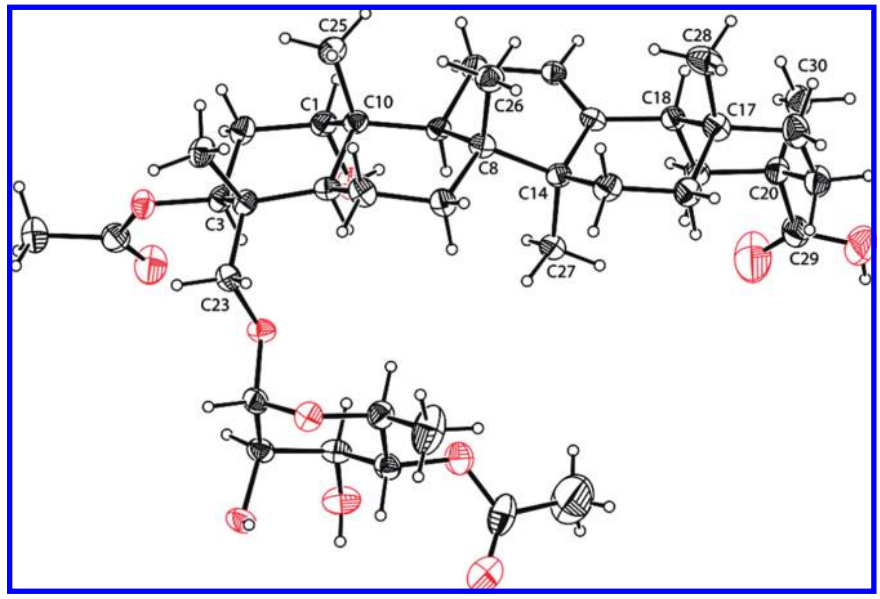


Figure 2. X-ray crystal structure with 30% probability ellipsoids for compound **4**.

were almost the same as those obtained for compound **4**, but slight differences suggested that the locations of the acetyl and sugar units were different. The absence of any acetyl group on the rhamnopyranose unit was suggested by the upfield shifts of protons H-3′ and H-4′ at δ_H 3.85 (dd, J = 9.3, 2.3 Hz) and 3.42 (t, J = 9.3 Hz), respectively. The locations of the two acetyl groups and the sugar unit at C-3, C-23, and C-1 were deduced from the correlations between H-3 and the carbonyl at δ_C 170.3, between H_2-23 and the carbonyl at δ_C 171.7, and between the anomeric proton and C-1 at δ_C 82.6, in the HMBC spectrum. Other NMR data confirmed that compound **5** is 1-*O*-[α-L-(rhamnopyranosyl]-23-acetoxy-3β-acetylimberbic acid. This is the first example of a pentacyclic triterpenoid glycosylated at position C-1.

Compound **6** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 713.4239, which suggested a sodiated molecular formula of $C_{39}H_{62}O_{10}Na$ (calcd 713.4241). Its spectroscopic data were closely comparable to those of compound **5**, but with only one methyl signal at δ_H 2.09, attributable to one acetoxy group, and the presence of an additional methoxy group at δ_H 3.65 and δ_C 52.1. The locations of the acetyl and methoxy groups at C-23 and C-29, and of the sugar unit at C-1, were deduced from correlations between CH_2-23 and the carbonyl at δ_C 171.9, between the methoxy protons and the carbonyl at δ_C 179.9, and between H-1′ and C-1 at δ_C 82.7, as observed in the HMBC spectrum. Other NMR data were used to confirm that compound **6** is the 29-methyl ester of 1-*O*-[α-L-(rhamnopyranosyl]-23-acetoxyimberbic acid.

Comparison of the experimental data (^1H and ^{13}C NMR and HR-MS data) with the literature showed **7** to correspond to 23-*O*-[α-L-(4′-acetylrhamnopyranosyl]imberbic acid, previously isolated from *C. imberbe*.[13]

Compound **8** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 755.4353, which suggested a sodiated molecular formula of $C_{41}H_{64}O_{11}Na$ (calcd 755.4346). The spectroscopic data were close to those of compound **6** but with signals for an additional acetyl group. The locations of the two acetyl and methoxy groups at C-3, C-23, and C-29, respectively, and of the sugar unit at C-1 were deduced from HMBC correlations between H-3 and the carbonyl at δ_C 170.9, between CH_2-23 and the carbonyl at δ_C 171.3, between the methoxy protons and the carbonyl at δ_C 179.7, and between H-1′ and C-1 at δ_C 81.3, as observed in the HMBC spectrum. Other NMR data confirmed that compound **8** is the 29-methyl ester of 1-*O*-[α-L-(rhamnopyranosyl]-23-acetoxy-3β-acetylimberbic acid.

Compound **9** was isolated as a colorless, amorphous powder, and the HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 755.4320, which suggested a sodiated molecular formula of $C_{41}H_{64}O_{11}Na$ (calcd 731.4346). The NMR data were closely comparable to those of compound **4** with an additional methoxy group at δ_H 3.62 and δ_C 52.0 attached at C-29, confirmed by the HMBC correlations from the methoxy protons to the carbonyl at δ_C 179.5. Compound **9** was assigned as the 29-methyl ester of 23-*O*-[α-L-(4′-acetylrhamnopyranosyl]-3β-acetylimberbic acid.

Compound **10** was isolated as a colorless, amorphous powder. The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 741.4178, which suggested a sodiated molecular formula of $C_{40}H_{62}O_{11}Na$ (calcd 741.4190), identical to compound **4**. The spectroscopic data of these two compounds were almost identical, but slight differences suggested that the locations of the acetyl and sugar units were different. The substitutions of the sugar moiety by two acetyl groups at positions 2′ and 4′ were deduced from the correlations observed in the HMBC spectrum from H-2′ to the carbonyl at δ_C 171.7 and from H-4′ to the carbonyl at δ 171.8 (Table 1). In addition, the resonances of the oxymethines, CH-1 and CH-3 at δ_H/δ_C 3.60/72.4 and δ_H/δ_C 4.09/66.1, respectively, confirmed the presence of hydroxy groups at these positions. The location of the sugar unit at C-23 was confirmed by the HMBC correlations from H-1′ to C-23. Compound **10** was assigned as 23-*O*-[α-L-(2′,4′-diacetylrhamnopyranosyl]imberbic acid.

The binding affinity of compounds **1**−**15** was evaluated by competition between Bcl-xL and the fluorescent tagged BH3 domain of the protein Bak, as described earlier.[5] Results are given by the K_i value, the dissociation constant of a compound determined at equilibrium with a reference ligand (Table 3). Compounds **1**, **5**, **10**, **14**, and **15** exhibited K_i values between 5.3 and 17.8 μM, indicating that they all act as antagonists of the Bcl-xL/Bak association. Several of these compounds also showed cytotoxic activity in various cancer cell lines with IC_{50} values within the μM range. Some very interesting observations can be summarized from these results. Most of the compounds from the first series (**1**−**10**) exhibited significant cytotoxicity, but only three compounds (**1**, **5**, and **10**) showed binding affinities for Bcl-xL, suggesting a different mechanism of action for compounds **2**−**4** and **6**−**9**. The glycosylated triterpenoids having a 29-methyl ester group (**6**, **8**, and **9**) exhibited the strongest cytotoxic activity, but did not show any binding activity for Bcl-xL. Since the methyl ester compound **2** and the glycosylated compound **5** are less cytotoxic, it could be deduced that the presence of the glycoside moiety together with

Table 3. Bcl-xL Binding Affinity and Cytotoxic Activity of Compounds **1**−**15** (K_i and IC_{50} in μM)

	Bcl-xL	cell line (IC_{50})			
compound	(K_i)	KB	HCT-116	MCF7	L1210
1[a]	17.8	5.4	4.7	6.0	NT[c]
2	>100	2.9	1.7	4.6	NT
3	>100	1.7	1.1	1.5	NT
4	>100	2	2.2	2.5	NT
5[a]	7.2	8.2	8	10.5	NT
6	>100	0.7	0.4	0.5	NT
7	>100	11.6	32.7	24.7	NT
8	>100	0.9	0.3	0.6	NT
9	>100	0.7	0.4	0.6	NT
10[b]	14.1	4.7	11.0	9.2	NT
11 + **13**	>100	15.8	41.8	44.7	16.3
12	>100	25.3	11.4	44.0	16.1
14[b]	7.6	15	5.8	11.3	6.8
15[b]	5.3	35.5	11.4	42.5	12.3
Taxotere (nM)		0.15	1.2	0.7	

[a] Triplicate runs. [b] Duplicate runs (Bcl-xL). [c] NT: not tested.

the methyl ester play an important role in the cytotoxic activity. In addition, it is suggested from these results for compounds having an acetylated rhamnose unit at position 23 (**3**, **4**, **7**, **9**, and **10**) that the acetyl group at position C-3 plays an important role in the cytotoxic activity (compounds **7** and **10**, less cytotoxic) but proved deleterious for Bcl-xL binding affinity (compounds **3**, **4**, and **9** were not active), on one hand, and that the presence of an acetyl group at C-2′ is found necessary for good binding affinity (cf. **7** and **10**) for the antiapoptotic protein Bcl-xL, on the other hand. In the second series (**11**−**15**), the compounds displayed weak to moderate cytotoxic activities for the four cancer cell lines (Table 3), but the presence of a primary alcohol at position 23 was found essential for a potent Bcl-xL binding affinity. It has been shown that lantadene A (**11**) induces efficient cell apoptosis for the HL-60 cancer cell line by activating the caspase-3 pathway and through down- and up-regulation of Bcl-2 and Bax expression, respectively.[19] The present results demonstrate that *L. camara* not only contains compounds interacting with the bcl-2 family of genes involved in apoptosis but also possesses compounds having binding affinity for the antiapoptotic Bcl-xL protein capable of disrupting the Bcl-xL/Bak association.

Experimental Section

General Experimental Procedures. Optical rotations were measured at 25 °C on a JASCO P1010 polarimeter. The UV spectra were recorded on a Perkin-Elmer Lamba 5 spectrophotometer. IR spectra were obtained on a Nicolet FTIR 205 spectrophotometer. The NMR spectra were recorded on a Bruker 500 MHz (Avance 500) spectrometer with $CDCl_3$ as solvent. HRESIMS were run on a MALDI-TOF spectrometer (Voyager-De STR; Perspective Biosystems). Kromasil analytical, semipreparative, and preparative C_{18} columns (250 × 4.5 mm; 250 × 10 mm and 250 × 21.2 mm i.d, 5 μm Thermo) were used for preparative HPLC separations using a Waters autopurification system equipped with a binary pump (Waters 2525), a UV−vis diode array detector (190−600 nm, Waters 2996), and a PL-ELS 1000 ELSD Polymer Laboratory detector. Analytical TLC plates (Si gel 60 F 254) were purchased from SDS (France). Polyamide DC 6 and polyamide cartridges were purchased from Macherey-Nagel (Chromabond PA, 1 g). Versapack C_{18} cartridges (40 × 75 mm) were purchased from Serlabo Technologies. All other chemicals and solvents were of analytical grade and purchased from SDS (France).

Plant Material. Leaves and flowers of *Combretum sundaicum* were collected in Bac Son, Lang Son Province, Vietnam, in July 1997 by Dr. D. Bastien, under the reference VN-266, and leaves of *Lantana camara* were collected in Vo Nhai, Vietnam, by Dr. A. Gramain, under the reference VN-528. These plants were identified by Dr. Q. B. Nguyen (Institute of Ecology), VAST, Hanoi, Vietnam. Herbarium specimens have been deposited at the Institute of Ecology (VAST, Hanoi).

Extraction and Isolation. Powdered leaves (1.38 kg) and flowers (115 g) of *C. sundaicum* were extracted with EtOAc (3 L × 5), and EtOAc and MeOH (250 mL × 5), respectively, at 40 °C. In turn, powdered leaves of *L. camara* (200 g) were extracted with a Dionex automated extractor using EtOAc and then MeOH (four runs each, 100 bar, 40 °C). The extracts were concentrated in vacuo at 40 °C to yield 26.3, 5.1, 6.4, and 4.6 g residues, respectively. A 100 mg aliquot of each crude extract was dissolved in AcOEt−MeOH (1:1) and filtered on a polyamide cartridge before being tested at 10 $\mu g \cdot mL^{-1}$ against the Bcl-xL protein in vitro. The filtered extract was then fractionated on a semipreparative C_{18} column according to a standardized method previously detailed.[21] Fractions 6 (*C. sundaicum* extract) and 5 (*L. camara* extract) were shown to possess binding affinity for Bcl-xL.

The EtOAc extract of *C. sundaicum* leaves (22.1 g) was submitted to silica gel chromatography using a gradient of heptane−CH_2Cl_2−MeOH (80:20:0 to 0:0:100), giving 15 fractions (FrA to FrO). Subsequent silica gel chromatography using the same gradient was performed on FrL, FrM, and FrN, with semipreparative HPLC finally used to purify compounds. From FrL (heptane−CH_2Cl_2−MeOH, 0:70:30), compounds **1** (30.5 mg, t_R 34.3 min), **8** (3.8 mg), and **9** (13.2 mg, t_R 37.0 min) were obtained with a mobile phase consisting of H_2O−MeCN (20:80). From FrM (heptane−CH_2Cl_2−MeOH, 0:50:50), compounds **3** (55.7 mg, t_R 31.0 min), **8** (24.6 mg, t_R 22.8 min), and **9** (11.5 mg) were obtained using the H_2O−MeCN mobile phase (15:85). From FrN (heptane−CH_2Cl_2−MeOH, 0:30:70), compounds **5** (38.9 mg, t_R 21.3 min) and **10** (13.4 mg, t_R 24.3) were obtained using the H_2O−MeCN mobile phase (20:80), and compounds **5** (35 mg) and **7** (22.7 mg, t_R 18.6 min) were obtained using a MeCN−H_2O gradient (50:50 to 100:0 in 20 min). Crystals of compound **4** (13 mg) were obtained from a CH_2Cl_2−MeOH−EtOAc mixture left at room temperature overnight.

The EtOAc and MeOH extracts of *C. sundaicum* flowers (5.1 g) and EtOAc and MeOH extracts of *L. camara* leaves (6.3 and 4.5 g) were submitted to flash chromatography on a reversed-phase column (Versapack C_{18} cartridge, 40 × 75 mm) using a step gradient mobile phase (solvent: A, MeCN, B, H_2O, 20−100% A for 70 min; flow rate: 10 mL/min) to give 10 fractions (FrA′ to FrJ′, FrA″ to FrJ″, and FrA‴ to FrJ‴, respectively). Silica gel chromatography using heptane−CH_2Cl_2−MeOH (80:20:0 to 0:0:100) was performed on FrG′, and preparative HPLC was used to purify the compounds present. Compounds **1** (12.7 mg, t_R 13.4 min), **2** (3.7 mg, t_R 14.5 min), **3** (10.5 mg, t_R 17.1 min), **4** (60.1 mg, t_R 16.7 min), and **5** (24.3 mg, t_R 9.1 min) were purified using preparative HPLC with the H_2O−MeCN mobile phase (5:95), while compound **6** (4.1 mg, t_R 18.1 min) was obtained with the H_2O−MeCN mobile phase (20:80). From FrF″ + FrF‴, compounds **14** (49.6 mg, t_R 32.0 min) and **15** (95.2 mg, t_R 34.0 min) were purified using preparative HPLC (250 × 19 mm i.d, 10 μm Sunfire) with the H_2O−MeCN mobile phase (35:65 + 0.1% TFA) at 17 $mL \cdot min^{-1}$ and compounds **11** and **13** (102.2 mg, t_R 29.8 min) and **12** (58.2 mg, t_R 32.3 min) with the H_2O−MeCN mobile phase (20:80 + 0.1% TFA).

23-Acetoxy-3*β*-acetylimberbic acid (1): white, amorphous powder; $[\alpha]^{25}_D$ +47.2 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 236 (2.7) nm; IR ν_{max} (ns) 2944, 1716, 1466, 1369 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 595.3583 $[M + Na]^+$ (calcd for $C_{34}H_{52}O_7Na$, 595.3611).

23-Acetoxyimberbic acid 29-methyl ester (2): colorless, amorphous powder; $[\alpha]^{25}_D$ +7.2 (*c* 0.37, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 236 (2.7) nm; IR ν_{max} (ns) 2947, 1723, 1471, 1352 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 567.3685 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_6Na$, 567.3662).

23-*O*-[α-L-(3′,4′-Diacetylrhamnopyranosyl]-3*β*-acetylimberbic acid (3): colorless, amorphous powder; $[\alpha]^{25}_D$ +18.4 (*c* 0.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 236 (2.8) nm; IR ν_{max} (ns) 3456, 2932, 1715, 1368 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 783.4280 $[M + Na]^+$ (calcd for $C_{42}H_{64}O_{12}Na$, 783.4295).

23-*O*-[α-L-(4′-Acetylrhamnopyranosyl]-3*β*-acetylimberbic acid (4): colorless crystals; $[\alpha]^{25}_D$ +29.9 (*c* 0.11, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 240 (2.7) nm; IR ν_{max} (ns) 3435, 2931, 1722, 1371 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 741.4180 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{11}Na$, 741.4190).

1-*O*-[α-L-(Rhamnopyranosyl]-23-acetoxy-3*β*-acetylimberbic acid (5): colorless, amorphous powder; $[\alpha]^{25}_D$ +54.2 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 239 (2.5) nm; IR ν_{max} (ns) 3418, 2928, 1714, 1371 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 741.4219 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{11}Na$, 741.4190).

1-*O*-[α-L-(Rhamnopyranosyl]-23-acetoxyimberbic acid 29-methyl ester (6): colorless, amorphous powder; $[\alpha]^{25}_D$ +27.9 (*c* 0.19, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 236 (2.7) nm; IR ν_{max} (ns) 3427, 2945, 1714, 1382 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 713.4239 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{10}Na$, 713.4241).

1-*O*-[α-L-(Rhamnopyranosyl]-23-acetoxy-3β-acetylimberbic acid 29-methyl ester (8): colorless, amorphous powder; $[\alpha]^{25}_D$ +51.3 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 236 (2.8) nm; IR ν_{max} (ns) 3445, 2931, 1722, 1370 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 755.4343 $[M + Na]^+$ (calcd for $C_{41}H_{64}O_{11}Na$, 755.4346).

23-*O*-[α-L-(4′-Acetylrhamnopyranosyl]-3β-acetylimberbic acid 29-methyl ester (9): colorless, amorphous powder; $[\alpha]^{25}_D$ +41.2 (*c* 0.09, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 237 (2.9) nm; IR ν_{max} (ns) 3448, 2943, 1722, 1371 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS *m/z* 755.4320, $[M + Na]^+$ (calcd for $C_{41}H_{64}O_{11}Na$, 755.4346).

23-*O*-[α-L-(2′,4′-Diacetylrhamnopyranosyl]imberbic acid (10): colorless, amorphous powder; $[\alpha]^{25}_D$ +31.2 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 237 (2.8) nm; IR ν_{max} (ns) 3454, 2939, 1727, 1373 cm^{-1}; ^1H NMR and ^{13}C NMR (Tables 1 and 2); HRESIMS, *m/z* 741.4178 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{11}Na$, 741.4190).

Crystal Data of 4.[25] The data were collected on an Enraf-Nonius kappaCCD diffractometer at 293(2) K using graphite-monochromated Mo Kα radiation ($\lambda = 0.71069$ Å). The structure was solved by direct methods using SHELXS-97[26] and refined by full-matrix least-squares on F^2 using SHELXL-97.[26] The asymmetric unit of the crystal consists of one molecule of **4** developing inter O−H···O bonds with two neighboring molecules along the 2-fold screw axis [distances between O1 and $O2^{i}$, and O4′ and $O3^{ii}$ are 2.724(5) and 2.815(6) Å, respectively, with symmetry codes i: *x*, 1+*y*, *z*; ii: 1−*x*, *y*−1/2, 1−*z*]. The structure calculated at room temperature shows peripheral side groups and side chains with somewhat important thermal disorder that could not be significantly improved by the joint use of DELU and SIMU restraints available in SHELXL-97. The crystal structure contains solvent-accessible voids of 107.0 $Å^3$, which can accommodate a disordered methanol molecule in the vicinity of the carboxylic group in C-29. The presence of residual density, which could not be modeled, was verified with PLATON/SQUEEZE[27] and is consistent with a quarter molecule of MeOH per asymmetric unit. The number of residual electrons thus located (9 per unit cell) was included in the formula, formula weight, calculated density, μ, and $F(000)$. The crystallographic data are summarized as follows: colorless prism of dimensions 0.50 × 0.25 × 0.22 mm, $C_{40}H_{62}O_{11}$, 0.25 (CH_4O), $M_r = 726.90$, monoclinic system, space group $P2_1$, $Z = 2$, $a = 14.035(3)$ Å, $b = 8.187(1)$ Å, $c = 18.977(3)$ Å, $\beta = 101.692(5)°$, $V = 4911.3(19)$ $Å^3$, $D_{calcd} = 1.131$ g/cm^3, $F(000) = 789$, $\mu = 0.080$ mm^{-1}, 20 367 collected reflections ($1.10° \le \theta \le 23.44°$), $-15 \le h \le 15$, $-8 \le k \le 9$, $-21 \le l \le 20$), 3030 independent reflections ($R_{int} = 0.0299$), goodness-of-fit on F^2 $S = 1.055$, $R_1 = 0.0699$ and $wR_2 = 0.1842$ for all reflections, $R_1 = 0.0595$ and $wR_2 = 0.1734$ for 2699 observed reflections [$I > 2\sigma(I)$], refining 468 parameters and 16 restraints, semiempirical absorption correction from multi φ- and ω-scans ($T_{min} = 0.87$, $T_{max} = 0.98$), final electron density between −0.211 and 0.239 e $Å^{-3}$.

Biological Assays. The binding affinity of compounds **1**−**15** was evaluated on Bcl-xL by competition against a fluorescent labeled reference compound (fluorescent tagged BH3 domain of the protein Bak), as described by Qian et al.[5] The unlabeled peptide Bak(BH3) and the substituted terphenyl derivative (compound number **4** in Kutzki et al.[28]) were used as positive control, with $K_i = 0.9$ and 0.81 μM, respectively. Results are expressed as binding activity, i.e., percentage of inhibition of the binding of labeled reference compound, or as K_i, the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to Cheng and Prusoff.[29]

The human KB tumor (oral epidermoid carcinoma), human colon cancer (HCT-116), breast cancer (MCF-7), and L1210 (mouse lymphocytic leukemia) cell lines were obtained originally from ATCC (Manassas, VA). The cytotoxicity assays were performed according to a published procedure.[30] Taxotere was used as reference compound.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**6** and **8**−**10** are available free of charge via the Internet at http://pubs.acs.org.

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