Potential Anticancer Activity of Naturally Occurring and Semisynthetic Derivatives of Aculeatins A and B from *Amomum aculeatum*[#]

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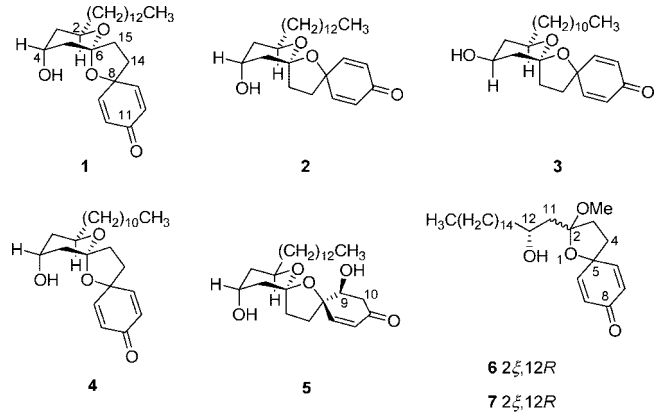
Activity-guided fractionation of hexanes- and $CHCl_3$-soluble extracts of *Amomum aculeatum* leaves, collected in Indonesia, led to the isolation of three new dioxadispiroketal-type (**3**–**5**) and two new oxaspiroketal-type (**6** and **7**) derivatives. Nine semisynthetic derivatives (**1a**−**1h** and **2a**) of the parent compounds, aculeatins A (**1**) and B (**2**), were prepared. All isolates and semisynthetic compounds were tested against a small panel of human cell lines. Of these, aculeatin A (**1**; ED_{50} 0.2–1.0 μM) was found to be among the most cytotoxic of the compounds tested and was further evaluated in an in vivo hollow fiber assay; it was found to be active against MCF-7 (human breast cancer) cells implanted intraperitoneally at doses of 6.25, 12.5, 25, and 50 mg/kg. However, when **1** was tested using P388 lymphocytic leukemia and human A2780 ovarian carcinoma in vivo models, it was deemed to be inactive at the doses used.

Amomum aculeatum Roxb. (Zingiberaceae) is a herbaceous plant, distributed in Indonesia, Malaysia, and Papua New Guinea, and has been used as a folk medicine for the treatment of fever and malaria.[1] In initial phytochemical work on this species, the 1,7-dioxadispiro[5.1.5.2]pentadecane-type compounds, aculeatins A−D, were characterized and found to exhibit antibacterial, antiprotozoal, and cytotoxic activities.[2,3] Of these compounds, aculeatins A, B, and D have been chemically synthesized due to their unusual structures and interesting bioactivities, and through enantioselective synthetic methods, the absolute configurations of these three substances were resolved definitively.[4–9]

In a preliminary short report, we have described the isolation, structural characterization, and cytotoxicity testing of aculeatols A−D, four C-9-hydroxylated analogues, from the leaves of *A. aculeatum*.[10] Aculeatols A−D are of structural and spectroscopic interest since they contain five stereocenters, as opposed to only three in the parent compounds, aculeatins A (**1**) and B (**2**), which were also present in the plant samples investigated.[10]

In the present study, as part of a collaborative approach to the discovery of new anticancer agents of plant origin,[11] we have delved more fully into the potential as cancer chemotherapeutic agents of compounds based on the unusual 1,7-dioxadispiro[5.1.5.2]pentadecane chemotype. Five new constituents (**3**−**7**) of *A. aculeatum* have been isolated and characterized and are reported herein. Since aculeatins A (**1**) and B (**2**) were isolated in reasonably high yields, these compounds have been derivatized to produce compounds **1a**−**1h** and **2a**, respectively, in an attempt to generate biologically potent analogues. Isolates (**3**−**7**) and derivatives (**1a**−**1h** and **2a**) were then evaluated against a small panel of human cancer cell lines. In addition, follow-up testing has been performed on aculeatin A (**1**) in an in vivo hollow fiber assay and also in P388 lymphocytic murine leukemia and human A2780 ovarian carcinoma in vivo models.



Results and Discussion

By bioactivity-guided fractionation of the hexanes- and chloroform-soluble extract of *A. aculeatum* leaves, using MCF-7 (human breast cancer) cells as a monitor, three new 1,7-dioxadispiro[5.1.5.2]pentadecane-type compounds (**3**−**5**) and two new 1-oxaspiro[4,5]decane-type compounds (**6** and **7**) were isolated and characterized.

Compound **3** was isolated as a yellow oil and displayed a sodiated molecular ion peak at *m/z* 413.2668 in the HRESIMS, corresponding to an elemental formula of $C_{24}H_{38}O_4Na$ (calcd for $C_{24}H_{38}O_4Na$, 413.2668). The ^1H NMR spectrum of **3** was closely comparable to that of aculeatin B (**2**),[2,10] suggesting the presence of a 1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one unit from signals in the range δ_H 1.28 to 6.99 and a long-chain aliphatic group from resonances between δ_H 0.88 and 1.47 (Table 1). However, the signals of **3** at δ_H 3.85 (1H, tt, J = 11.2, 4.4 Hz, H-4) and 3.36 (1H, m, H-2) appeared in a more upfield region when compared with analogous signals of aculeatin B [H-4 (δ_H 4.35) and H-2 (δ_H 3.86)]. These differences suggested that compound **3** has a different configuration at the C-4 position from aculeatin B (**2**). The coupling constants of 11.2 and 4.4 Hz for H-4 implied an axial position of this proton in the cyclohexane ring system, as opposed to the equatorial orientation in **2** (3.0 Hz, H-4).[10] From the 2D NOESY data of **3**, it was found that H-4 exhibited NOE correlations with H-2 and H-15b (Figure 1), which showed the same orientation for both H-2 and H-4, and was used to establish the *R* and *S* configurations of C-2 and C-4, respectively. The configuration of

[#] Dedicated to Dr. G. Robert Pettit for his pioneering work on bioactive natural products.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **3** and **5**

	aculeatin E (**3**)		aculeatol E (**5**)	
position	δ_C, mult.[a]	δ_H (J in Hz)	δ_C, mult.[a]	δ_H (J in Hz)
2	71.6, CH	3.36, m	69.7, CH	3.91, m
3	40.8, CH_2	1.96, m	37.8, CH_2	1.61, m
		1.23, m		1.51, m
4	66.8, CH	3.85, tt (11.2, 4.4)	65.3, CH	4.38, m
5	43.5, CH_2	2.12, m	40.3, CH_2	2.03, dd (13.9, 3.1)
		1.78, m		1.86, m
6	109.2, qC		106.7, qC	
8	78.0, qC		81.9, qC	
9	148.9, CH	6.79, dd (10.0, 2.9)	69.7, CH	3.93, dd (8.7, 5.4)
10	127.1, CH	6.13, dd (10.0, 1.8)	44.2, CH_2	2.73, m
11	185.5, qC		198.6, qC	
12	127.3, CH	6.14, dd (10.4, 1.8)	129.6, CH	5.99, d (10.0)
13	151.6, CH	6.99, dd (10.4, 2.9)	148.0, CH	6.61, d (10.0)
14	34.9, CH_2	2.28, m	31.7, CH_2	2.52, dd (11.7, 8.2)
		2.06, m		1.86, m
15	33.3, CH_2	2.39, m	34.6, CH_2	2.65, dd (12.2, 8.2)
		1.83, m		1.87, m
16	35.7, CH_2	1.62, m, 1.52, m	35.7, CH_2	1.57, m 1.44, m
17	25.8, CH_2	1.47, m	25.5, CH_2	1.44, m
		1.31, m		1.30, m
18–25	29.3–29.6, CH_2	1.26–1.30, m	29.4–29.7, CH_2	1.23–1.30, m
26	31.9, CH_2	1.28, m	31.9, CH_2	1.23–1.30, m
27	22.6, CH_2	1.28, m	22.7, CH_2	1.26, m
28	14.1, CH_3	0.88, t (6.6)	14.1, CH_3	0.89, t (7.0)

[a] Multiplicity was deduced from the DEPT and HSQC spectroscopic data.

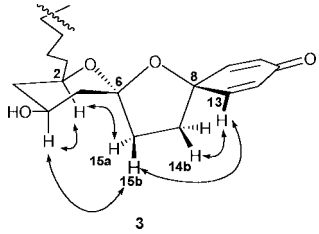
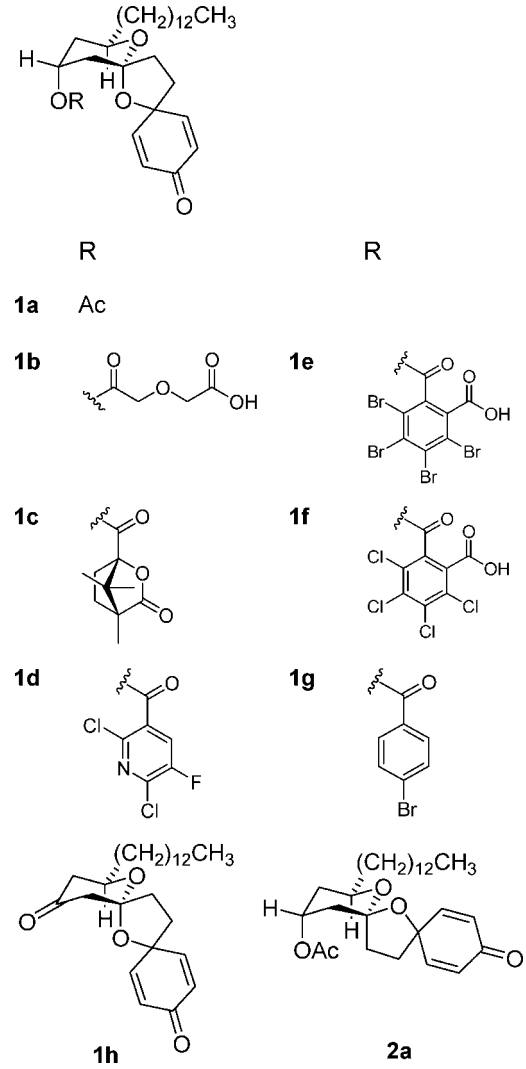


Figure 1. Key NOESY correlations of **3**.

the C-6 position was confirmed as *S* by the observation of a NOE correlation between H-2 and H-15a. When all of the spectroscopic data were taken into account, the structure of compound **3** was determined as 2*R**,4*S**,6*S**-4-hydroxy-2-undecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one, and this isolate has been named aculeatin E.

The molecular formula of compound **4** was assigned as $C_{24}H_{38}O_4$ and was inferred from a sodiated molecular ion peak at *m*/*z* 413.2672 (calcd for $C_{24}H_{38}O_4Na$, 413.2668) in the HRESIMS. The ^{1}H NMR spectrum of **4** disclosed resonances at δ_H 6.86 (1H, dd, J = 10.0, 2.9 Hz, H-13), 6.77 (1H, dd, J = 9.9, 2.9 Hz, H-9), 6.15 (1H, dd, J = 10.0, 1.8 Hz, H-12), 6.11 (1H, dd, J = 9.9, 1.8 Hz, H-10), 4.13 (2H, m, H-2 and H-4), 2.37 (1H, m, H-14a), 2.24 (1H, m, H-15a), 2.00 (3H, m, H-5a, H-14b, and H-15b), 1.94 (1H, m, H-5b), 1.80 (1H, brd, J = 13.1 Hz, H-3a), 1.50 (2H, m, H-16), 1.43 (1H, m, H-17a), and 1.41 (1H, dd, J = 13.1, 2.6 Hz, H-3b), assignable to the same dioxadispiroketal-type skeleton as aculeatin A (**1**).[2] Comparison of the ^{1}H and ^{13}C NMR chemical shifts of **4** with those of aculeatin A indicated that both compounds have identical carbon skeletons. From detailed NMR assignments, the relative configurations at the chiral carbons in **4** were confirmed as being the same as aculeatin A (**1**) by the observation of correlations in several 2D NMR (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) experiments. The only difference was observed in the respective molecular formula of these substances. As shown by the MS data, compound **4** has two less methylene units in the aliphatic side chain when compared with aculeatin A ($C_{26}H_{42}O_4$). Thus, the structure of **4** (aculeatin F) was determined as 2*R**,4*R**,6*R**-4-hydroxy-2-undecyl-1,7-dioxadispiro[5.1.5.2]pentadec-9,12-dien-11-one.



The ^{1}H NMR spectroscopic data (Table 1) of compound **5** exhibited three oxymethine signals at δ_H 4.38 (1H, m, H-4), 3.93

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **6** and **7**

	amomol A (**6**)		amomol B (**7**)	
position	δ_C, mult.[a]	δ_H (J in Hz)	δ_C[a]	δ_H (J in Hz)
2	111.6, qC		111.5	
3	36.6, CH_2	2.34, m 2.15, m	36.6	2.24, m
4	34.9, CH_2	2.39, m	35.1	2.43, m
		2.07, m		2.07, m
5	79.1, qC		78.7	
6	148.4, CH	6.78, d (10.0, 2.7)	148.2	6.81, d (10.0)
7	127.1, CH	6.13, d (10.0)	127.4[b]	6.14, d (10.0)
8	185.2, qC		185.3	.
9	127.7, CH	6.17, d (10.0)	127.5[b]	6.17, d (10.5)
10	150.6, CH	6.85, d (10.0, 2.7)	150.6	6.85, d (10.5)
11	40.7, CH_2	2.13, m	40.1	1.96, m
		1.70, brd (14.7)		1.96, m
12	69.1, CH	3.84, m	68.2	3.79, m
13	37.8, CH_2	1.54, m	37.8	1.53, m
		1.43, m		1.43, m
14	25.4, CH_2	1.43, m,	25.5	1.43, m,
		1.33, m		1.33, m
15–24	29.6–29.7, CH_2	1.26–1.33, m	29.6–29.7	1.26–1.33, m
25	31.9, CH_2	1.26, m	31.9	1.26, m
26	22.7, CH_2	1.30, m	22.7	1.30, m
27	14.1, CH_3	0.88, t (6.8)	14.1	0.89, t (6.7)
OCH_3	48.9, CH_3	3.35, s	48.5	3.35, s

[a] Multiplicity was deduced from the DEPT and HSQC spectroscopic data. [b] Assignments are interchangeable.

(1H, dd, J = 8.7, 5.4 Hz, H-9), and 3.91 (1H, m, H-2), two olefinic proton signals at δ_H 6.61 (1H, d, J = 10.0 Hz, H-13) and 5.99 (1H, d, J = 10.0 Hz, H-12), and one methylene signal at δ_H 2.73 (2H, m, H-10), attributable to an aculeatol-type structure.[10] In addition, it was found that the ^{13}C NMR spectroscopic data of **5** were generally comparable to those of aculeatol D.[10] The ^{13}C NMR chemical shifts at δ_C 69.7 (C-2) and 34.6 (C-15) were supportive of the configuration of C-6 as being *S*.[10] The coupling constants of H-9 (8.7 and 5.4 Hz) and H-5a (13.9 and 3.1 Hz), and NOE correlations between H-2/H-15a, H-9/H-14a, and H-13/H-14b in the 2D NOESY spectrum of **5**, suggested that the relative configurations at the other chiral centers in this structure are the same as those of aculeatol D.[10] The distinction between compound **5** and aculeatol D is in the length of the alkyl side chain at C-2, as shown in the molecular formula of $C_{26}H_{44}O_5$ for **5** compared with $C_{24}H_{40}O_5$ for aculeatol D. Therefore, compound **5** (aculeatol E) was elucidated as 2*R**,4*R**,6*S**,8*R**,9*S**-4,9-dihydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one.

The HRESIMS of **6** provided a sodiated molecular ion peak at m/z 457.3294 $[M + Na]^+$, suggesting $C_{27}H_{46}O_4$ to be its molecular formula. The ^{1}H NMR spectrum of compound **6** exhibited resonances at δ_H 6.85 (1H, dd, J = 10.0, 2.7 Hz, H-10), 6.78 (1H, dd, J = 10.0, 2.7 Hz, H-6), 6.17 (1H, d, J = 10.0 Hz, H-9), 6.13 (1H, d, J = 10.0 Hz, H-7), 2.39 (1H, m, H-4a), 2.34 (1H, m, H-3a), 2.15 (1H, m, H-3b), and 2.07 (1H, m, H-4b), due to the presence of an oxaspiro[4,5]deca-6,9-dien-8-one unit, which has not been described in the literature to date (Table 2). From the remaining proton signals, only one oxymethine proton at δ_H 3.84 was observed when compared with two oxymethines present in aculeatin A (**1**), suggesting that the cyclohexane ring of a dioxadispiroketal-type compound has been cleaved in compound **6**. This assumption was supported by the observed HMBC correlation between the methoxy protons at δ_H 3.32 and the spiro carbon at δ_C 111.6 (C-2), instead of a correlation between H-14 and C-2 as exhibited by aculeatin-type compounds.[2,10] The proton resonance signals at δ_H 2.39 and 2.07 (H-4) exhibited three-bond correlations with C-6 and C-10, respectively, which enabled the H-3 and H-4 signals to be assigned unambiguously. The preparation of Mosher ester derivatives of **6** was used to provide the absolute configuration of C-12 as *R*, based on the differences $\Delta\delta_{R\text{-}S}$ +0.081 (H11a), +0.049 (H-11b), and −0.014 (H-13a) between the (*R*)-MTPA and (*S*)-MTPA esters of **6**.[12–15] Although the NOESY correlations of δ_H 6.82 (H-10) to 2.36 (H-4a) and 3.32 (OMe) and δ_H 6.75 (H-6) to 2.04 (H-4b) and 1.67 (H-11b) were observed, those correlations were not sufficient to demonstrate the relative configuration at the C-2 position. Thus, compound **6** (amomol A) was determined structurally as 2ξ,12*R*-2-(2-hydroxyheptadecyl)-2-methoxy-1-oxaspiro[4.5]deca-6,9-dien-8-one.

Compound **7** gave the same molecular formula ($C_{27}H_{46}O_4$) as compound **6**, and these compounds showed a close resemblance in their ^{1}H and ^{13}C NMR spectroscopic data (Table 2). Furthermore, by analysis of the 2D NMR (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) spectroscopic data, it was evident that the relative configuration of the 2-methoxy-1-oxaspiro[4.5]deca-6,9-dien-8-one unit of compound **7** was identical to that of **6**. On preparing (*R*)- and (*S*)-MTPA esters of compound **7**, the absolute configuration of C-12 in the alkyl side chain was established as *R*, the same configuration as in compound **6**. Since these two similar compounds were able to be separated on a nonchiral HPLC column and their optical rotations are of the opposite signs, the configuration of C-2 in compounds **6** and **7** was inferred as being opposite, suggesting that these compounds are isomers. Therefore, the structure of **7** (amomol B) was also designated as 2ξ,12*R*-2-(2-hydroxyheptadecyl)-2-methoxy-1-oxaspiro[4.5]deca-6,9-dien-8-one.

All isolated compounds were evaluated for their cytotoxic activity using a small panel of human cancer cell lines, and their cytotoxic activities are summarized in Table 3.[16,17] It was found that the five new compounds **3**–**7** were cytotoxic against the human cancer cell lines in which they were evaluated and comparable in potency to the parent compounds, aculeatins A (**1**) and B (**2**). Since aculeatin A (**1**) was isolated in a large quantity in the present study and exhibited potent cytotoxic activity, modifications in the structure of this parent compound were performed in an attempt to develop more active analogues. Eight semisynthetic compounds from aculeatin A, **1a**−**1h**, and aculeatin B acetate, **2a**, were synthesized. Modification of the free hydroxy group at the C-4 position in aculeatin A (**1**) resulted in decreased cytotoxic activities for these compounds, as shown in Table 3. Likewise, the same cytotoxic trend was evident for aculeatin B (**2**) even though only one analogue (**2a**) was tested in the present study. Thus, a free hydroxy group at the C-4 position in aculeatins A (**1**) and B (**2**) seems to be necessary for the elicitation of potent cytotoxic activity within this compound group.

Aculeatin A (**1**) was chosen for follow-up evaluation in an in vivo hollow fiber assay[18–21] due to its cytotoxicity in the human cancer cell panel and its availability in a reasonably large quantity.

Table 3. Cytotoxicity Data of Compounds **1**, **1a**−**1h**, **2**, **2a**, and **3**−**7**

	cell line[a,b]		
compound	Lu1	LNCaP	MCF-7
1	1.0	0.5	0. 2
1a	2.6	1.1	1.5
1b	3.4	1.9	1.3
1c	4.0	3.0	3.2
1d	22.2	17.9	14.6
1e	10.3	5.1	4.7
1f	12.8	5.8	4.8
1g	1.3	0.8	0.7
1h	30.5	22.1	7.5
2	3.1	1.2	1.9
2a	5.7	2.2	3.5
3	1.8	1.8	0.5
4	1.0	0.7	0.5
5	1.8	4.4	3.9
6	0.9	0.7	0.9
7	0.5	0.9	0.7

[a] Results are expressed as ED_{50} values (μM), and values < 10 μM are considered to be active. [b] Key to cell lines used: Lu1 (human lung carcinoma); LNCaP (hormone-dependent human prostate carcinoma); MCF-7 (human breast carcinoma).

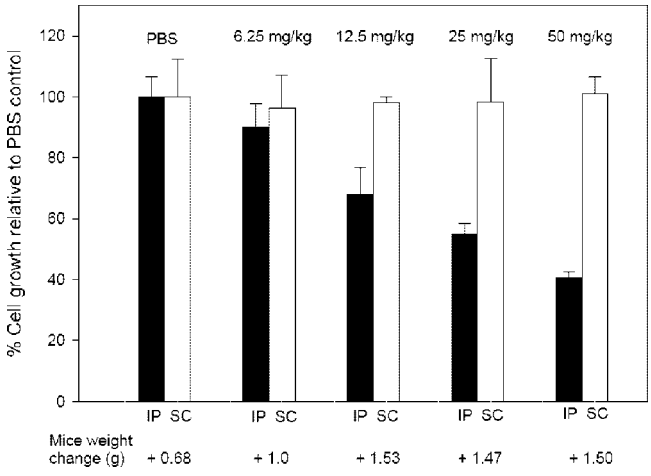


Figure 2. Effect of aculeatin A (**1**) on the growth of MCF-7 cells in the hollow fiber assay. Hollow fibers containing either Lu1, LNCaP, or MCF-7 cells were propagated subcutaneously (SC) or within the peritoneum (IP) of immunodeficient mice. The animals were treated with vehicle or aculeatin A (**1**) once daily by intraperitoneal injection (6.25, 12.5, 25, 50 mg/kg) from days 3–6 after implantation.

This procedure is used as a secondary bioassay in our program on the discovery of natural product anticancer agents, since it is predictive of efficacy in follow-up in vivo assays and does not consume much test compound.[20,21] Aculeatin A (**1**) inhibited the growth of the MCF-7 cell line (10–60%), propagated in hollow fibers via the intraperitoneal site, in the dose range 6.25–50 mg/kg (Figure 2). However, this compound was inactive when evaluated against the Lu1 (human lung cancer) and LNCaP (human prostate cancer) cells implanted at the intraperitoneal site at the same dose range (Figure S1, Supporting Information). Moreover, compound **1** was not active against any of the three cell lines used when implanted subcutaneously (dose range 6.25–50 mg/kg). When aculeatin A (**1**) was assessed in the in vivo P388 lymphocytic leukemia model,[22,23] by intraperitoneal injection (15 mg/kg/injection), it was found to be inactive (T/C = 122%) in this model. Furthermore, compound **1** was inactive in a human A2780S ovarian carcinoma xenograft mouse model[24,25] and gave 0.4 log cell kill at 3 mg/kg/injection. Accordingly, it seems that compounds of the 1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one structural type should only be investigated further as potential anticancer agents as low-priority leads. However, owing to the cytotoxic potency of aculeatin A (**1**) against the MCF-7 cell line and its efficacy against MCF-7 cells in hollow fibers, it may be worth performing a xenograft study on **1** with this same model.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer UV/vis lambda 10 spectrometer. IR spectra were run on a Nicolet Protégé 460 FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature using a Bruker DRX-400 spectrometer. Chemical shifts were reported as ppm with reference to the residual solvent or tetramethylsilane (TMS). Electrospray ionization (ESI) mass spectrometric analyses were obtained on a 3-T Finnigan FTMS-2000 Fourier transform mass spectrometer. A SunFire PrepC_{18}OBD column (5 μm, 150 × 19 mm i.d., Waters, Milford, MA) and a SunFire PrepC_{18} guard column (5 μm, 10 × 19 mm i.d., Waters) were used for preparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector (Waters). Column chromatography was carried out with Purasil (230–400 mesh, Whatman, Clifton, NJ) and Sephadex LH-20 (Sigma, St. Louis, MO). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thickness Partisil K6F (Whatman) glass plates, while preparative thin-layer chromatography was conducted on precoated 20 × 20 cm, 500 μm Partisil K6F (Whatman) glass plates. Semisynthetic reagents were purchased from Sigma (St. Louis, MO). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ).

Plant Material. The leaves of *A. aculeatum* Roxb. were collected at Gunung Kancana, West Java, Indonesia, in August 2003. The plant was identified by S.R. and A.R. A voucher specimen (SR-CJR 8) has been deposited at the Herbarium Bogoriense, Bogor, Indonesia.

Extraction and Isolation. The dried and milled leaves (803 g) of *A. aculeatum* were extracted with MeOH (3 × 1.5 L) overnight at room temperature. The solvent was evaporated in vacuo to afford a concentrated MeOH extract, which was then diluted with H_2O (0.9 L) to give an aqueous MeOH extract (1.0 L). This aqueous extract was partitioned in turn with hexanes (3 × 1.0 L), $CHCl_3$ (3 × 1.0 L), and EtOAc (3 × 1.0 L), to afford dried hexanes- (10 g), $CHCl_3$- (2.1 g), EtOAc- (6.6 g), and H_2O-soluble (21 g) residues. The hexanes- and $CHCl_3$-soluble extracts exhibited significant cytotoxicity against a human breast cancer cell line (MCF-7). Accordingly, the hexanes (ED_{50} = 1.0 μg/mL) and $CHCl_3$ (ED_{50} = 1.2 μg/mL) extracts were combined and subjected to silica gel column chromatography (10 × 40 cm, 230–400 mesh), eluted with pure $CHCl_3$ initially, then with a gradient mixture of $CHCl_3$−MeOH (100:1 to 1:1), to give eight fractions (F01−F08). These fractions were evaluated in the MCF-7 cell line, and the ED_{50} (μg/mL) values were >20, 0.3, 0.1, 0.9, 1.2, 5.0, 8.7, and >20, respectively. Fraction F02 (3.5 g) was chromatographed on a silica gel column (4 × 45 cm) eluted with hexanes−EtOAc−MeOH (50:10:1) to give aculeatin A[10] (**1**, 300 mg) and aculeatin B[10] (**2**, 12 mg). During purification of a portion (40 mg) of crude aculeatin A (300 mg) using HPLC separation (MeCN−H_2O = 90:10, 8 mL/min), aculeatin F (**4**, 10 mg, t_R 20 min) was isolated. Fraction F03 (1.25 g) was also subjected to silica gel chromatography (2.5 × 45 cm) eluted with hexane−EtOAc-MeOH (30:15:1 to 30:15:2) to give nine subfractions (F0301−0309). The constituents of subfraction F0306 were further purified by HPLC using a reversed-phase C_{18} column (H_2O−MeOH 85%−100% in 60 min) to furnish **3** (2.1 mg, t_R 24.6 min), **5** (1.3 mg, t_R 35.4 min), and a mixture of **6** and **7** (10.0 mg, t_R 57.5 min). Repetitive HPLC separation of this mixture using the same conditions afforded compounds **6** (4.6 mg, t_R 56.8 min) and **7** (3.2 mg, t_R 57.7 min).

Aculeatin E (3): yellow oil; $[\alpha]^{22}_D$ +5.1 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.18) nm; IR (film) ν_{max} 3440, 2923, 1671, 1634, 1080, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m/z* 413.2668 [M + Na], (calcd for $C_{24}H_{38}O_4Na$, 413.2668).

Aculeatin F (4): yellow oil; $[\alpha]^{22}_D$ −5.0 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.27) nm; IR (film) ν_{max} 3442, 2921, 1671, 1634, 1080, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.86 (1H, dd, J = 10.0, 2.9 Hz, H-13), 6.77 (1H, dd, J = 9.9, 2.9 Hz, H-9), 6.15 (1H, dd, J = 10.0, 1.8 Hz, H-12), 6.11 (1H, dd, J = 9.9, 1.8 Hz, H-10), 4.13 (2H, m. H-2 and H-4), 2.37 (1H, m, H-14a), 2.24 (1H, m, H-15a),

2.00 (3H, m, H-5a, H-14b, and H-15b), 1.94 (1H, m, H-5b), 1.80 (1H, brd, J = 13.1 Hz, H-3a), 1.50 (2H, m, H-16), 1.43 (1H, m, H-17a), 1.41 (1H, dd, J = 13.1, 2.6 Hz, H-3b), 1.30 (1H, m, H-17b), 1.23–1.27 (16H, m, H-18 to H-25), 0.88 (3H, t, J = 6.7 Hz, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.3 (C, C-11), 150.9 (CH, C-13), 148.7 (CH, C-9), 127.3 (CH, C-12), 127.0 (CH, C-10), 109.1 (C, C-6), 79.7 (C, C-8), 65.3* (CH, C-2), 64.8* (CH, C-4), 39.1 (CH_2, C-5 and C-15), 37.9 (CH_2, C-3), 35.9 (CH_2, C-16), 34.1 (CH_2, C-14), 31.9 (CH_2, C-24), 29.6–29.7 (CH_2, C-18 to C-22), 29.3 (CH_2, C-23), 25.6 (CH_2, C-17), 22.7 (CH_2, C-25), 14.1 (CH_3, C-26) (*assignments are interchangeable); HRESIMS m/z 413.2672 [M + Na] (calcd for $C_{24}H_{38}O_4Na$, 413.2672).

Aculeatol E (5): yellow oil; $[\alpha]^{22}_D$ +8.4 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (4.15) nm; IR (film) ν_{max} 3441, 2921, 1670, 1637, 1080, 1014 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS m/z 459.3092 [M + Na] (calcd for $C_{26}H_{44}O_5Na$, 459.3086).

Amomol A (6): amorphous, white powder; $[\alpha]^{22}_D$ −11.4 (c 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.16) nm; IR (film) ν_{max} 3442, 2921, 2851, 1671, 1634, 1080, 1014 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRESIMS m/z 457.3294 [M + Na]$^+$ (calcd for $C_{27}H_{46}O_4Na$, 457.3294).

Amomol B (7): amorphous, white powder; $[\alpha]^{22}_D$ +8.8 (c 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (3.98) nm; IR (film) ν_{max} 3442, 2917, 2850, 1673, 1634, 1081, 1017 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRESIMS m/z 457.3294 [M + Na]$^+$ (calcd for $C_{27}H_{46}O_4Na$, 457.3294).

Preparation of Mosher Ester Derivatives of 6 and 7. The (*R*)-MTPA and (*S*)-MTPA esters of **6** and **7** were prepared by a convenient Mosher ester method described in the literature.[15] **Compound 6a** [12-(*R*)-MTPA ester of **6**]: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.958 (1H, H-10), 6.931 (1H, H-6), 6.310 (1H, H-9), 6.281 (1H, H-7), 5.533 (1H, m, H-12), 3.389 (3H, OCH_3), 2.613 (1H, H-11a), 2.191 (1H, H-11b), 1.818 (2H, H-13), 1.393–1.191 (CH_2), 0.874 (3H, H-27); HRESIMS m/z 650.3794 [M + Na]$^+$ (calcd for $C_{37}H_{53}F_3O_6$, 650.3794). **Compound 6b** [12-(*S*)-MTPA ester of **6**]: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.945 (1H, H-10), 6.878 (1H, H-6), 6.292 (1H, H-9), 6.273 (1H, H-7), 5.531 (1H, H-12), 3.284 (3H, OCH_3), 2.532 (1H, H-11a), 2.142 (1H, H-11b), 1.832 (2H, H-13), 1.391–1.182 (CH_2), 0.875 (3H, H-27); HRESIMS m/z 650.3794 [M + Na]$^+$ (calcd for $C_{37}H_{53}F_3O_6$, 650.3794). **Compound 7a** [12-(*R*)-MTPA ester of **7**]: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.964 (1H, H-10), 6.916 (1H, H-6), 6.311 (1H, H-9), 6.251 (1H, H-7), 5.549 (1H, H-12), 3.332 (3H, OCH_3), 2.358 (2H, H-11), 1.780 (2H, H-13), 1.486–1.152 (CH_2), 0.875 (3H, H-27); HRESIMS m/z 650.3794 [M + Na]$^+$ (calcd for $C_{37}H_{53}F_3O_6$, 650.3794). **Compound 7b** [12-(*S*)-MTPA ester of **7**]: ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.963 (1H, H-10), 6.899 (1H, H-6), 6.304 (1H, H-9), 6.252 (1H, H-7), 5.487 (1H, H-12), 3.280 (3H, OCH_3), 2.355 (2H, H-11), 1.861 (2H, H-13), 1.481–1.198 (CH_2), 0.874 (3H, H-27); HRESIMS m/z 650.3794 [M + Na]$^+$ (calcd for $C_{37}H_{53}F_3O_6$, 650.3794).

Preparation of 1a. Aculeatin A (**1**) (10 mg) was acetylated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature for 24 h. The reaction product was purified by preparative TLC using hexanes−acetone (2:1) to give aculeatin A 4-acetate (**1a**, 7.0 mg, R_f 0.7): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.89 (1H, dd, J = 10.1, 3.0 Hz, H-13), 6.74 (1H, dd, J = 10.1, 3.0 Hz, H-9), 6.12 (1H, dd, J = 10.1, 2.0 Hz, H-12), 6.08 (1H, dd, J = 10.1, 2.0 Hz, H-10), 5.05 (1H, pentet, J = 2.8 Hz, H-4eq), 4.13 m (1H, H-2ax), 2.35 (1H, brt, J = 7.1 Hz, H-14a), 2.19 (1H, dd, J = 9.4, 7.1 Hz, H-15a), 2.14 (1H, td, J = 15.2, 2.3 Hz, H-5a), 2.00 (3H, s, $COCH_3$), 1.96 (1H, m, H-14b), 1.96 (1H, m, H-15b), 1.92 (1H, dd, J = 14.9, 3.8 Hz, H-5b), 1.76 (1H, brd, J = 14.2 Hz, H-3a), 1.47 (3H, m, H-16 and H-17a), 1.32 (1H, m, H-17b), 1.30 (1H, m, H-3b), 1.33–1.23 (22H, m, H-18 to H-27), 0.88 (3H, t, J = 6.8 Hz, H-28); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.8 (C, C-11), 171.2 (C, COO), 152.2 (CH, C-13), 149.8 (CH, C-9), 126.8 (CH, C-12), 126.6 (CH, C-10), 107.4 (C, C-6), 79.3 (C, C-8), 67.2 (CH, C-4), 65.5 (CH, C-2), 39.5 (CH_2, C-15), 35.9 (CH_2, C-5), 35.9 (CH_2, C-16), 34.8 (CH_2, C-3), 34.1 (CH_2, C-14), 31.9 (CH_2, C-26), 29.7–29.6 (CH_2, C-18 to C-24), 25.6 (CH_2, C-17), 29.4 (CH_2, C-25), 22.7 (CH_2, C-27), 21.5 (CH_3, $OCOCH_3$), 14.1 (CH_3, C-28); HRESIMS m/z 483.3083 [M + Na]$^+$ (calcd for $C_{28}H_{44}O_5Na$, 483.3080).

Preparation of 1b. Compound **1** (10 mg) was treated with diglycolic anhydride (10 mg) and pyridine (0.5 mL) at room temperature for 24 h. The reaction product was purified by preparative TLC (hexanes−EtOAc = 2:1) to give compound **1b** (7 mg, R_f 0.3): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (1H), 6.74 (1H), 6.13 (1H), 6.09 (1H), 5.17 (1H), 4.10 (1H), 4.17 (4H), 2.35 (1H), 2.20 (1H), 2.20 (1H), 1.99 (1H), 1.99 (1H), 1.97 (1H), 1.80 (1H), 1.53 (1H), 1.48 (2H), 1.48 (1H), 1.34 (1H), 1.33–1.23 (20H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.9 (C), 172.5 (C), 170.5 (C), 151.9 (CH), 149.5 (CH), 127.0 (CH), 126.8 (CH), 107.2 (C), 79.3 (C), 68.8 (CH), 68.8 (CH_2) 65.5 (CH), 39.4 (CH_2), 35.8 (CH_2), 35.7 (CH_2), 34.6 (CH_2), 34.0 (CH_2), 31.9 (CH_2), 29.7–29.6 (CH_2), 29.4 (CH_2), 25.6 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HRESIMS m/z 557.3109 [M + Na]$^+$ (calcd for $C_{30}H_{46}O_8Na$, 557.3085).

Preparation of 1c. Compound **1** (10 mg) was treated with (*S*)-campanoyl chloride (10 mg) and 4-(dimethylamino)pyridine (10 mg) in pyridine (1.0 mL) at room temperature for 48 h. The reaction product was purified by preparative TLC (hexanes−EtOAc = 2:1) to give compound **1c** (8 mg, R_f 0.5): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (1H), 6.77 (1H), 6.15 (1H), 6.11 (1H), 5.23 (1H), 4.13 (1H), 2.39 (1H), 2.23 (1H), 2.18 (1H), 2.01 (1H), 1.99 (1H), 1.98 (1H),1.84 (1H), 1.57 (1H), 1.47 (2H), 1.47 (1H), 1.33 (1H), 1.33–1.23 (20H), 1.06 (3H), 0.97 (3H), 0.92 (3H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.4 (C), 178.1 (C), 167.0 (C), 151.7 (CH), 149.3 (CH), 126.9 (CH), 126.5 (CH), 107.0 (C), 91.3 (C), 79.1 (C), 69.0 (CH), 65.4 (CH), 54.8 (C), 53.8 (C), 39.2, (CH_2), 35.9 (CH_2), 35.7 (CH_2), 34.6 (CH_2), 33.9 (CH_2), 31.9 (CH_2), 30.4 (CH_2), 29.6–29.5 (CH_2), 29.3 (CH_2), 28.7 (CH_2), 25.5 (CH_2), 22.6 (CH_2), 16.9 (CH_3), 16.8 (CH_3), 14.1 (CH_3), 9.6 (CH_3); HRESIMS m/z 621.3774 [M + Na]$^+$ (calcd for $C_{36}H_{54}O_7Na$, 621.3762).

Preparation of 1d. Compound **1** (10 mg) was treated with 2,6-dichloro-5-fluoropyridinecarbonyl chloride (15 mg) and 4-(dimethylamino)pyridine (10 mg) in pyridine at room temperature for 48 h. The reaction product was purified by preparative TLC (hexanes−EtOAc = 2:1) to give compound **1d** (9 mg, R_f 0.6): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (1H), 6.90 (1H), 6.67 (1H), 6.19 (1H), 6.07 (1H), 5.40 (1H), 4.18 (1H), 2.43 (1H), 2.28 (1H), 2.18 (1H), 2.07 (1H), 2.03 (1H), 2.03 (1H), 1.91 (1H), 1.62 (1H), 1.50 (1H), 1.50 (1H), 1.34 (1H), 1.33–1.23 (20H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.4 (C), 161.5 (C), 154.9 (C), 152.3 (C), 150.9 (CH), 148.4 (CH), 127.4 (CH), 127.1 (CH), 107.6 (C), 79.2 (C), 69.4 (CH), 65.7 (CH), 39.3 (CH_2), 36.1 (CH_2), 35.8 (CH_2), 34.4 (CH_2), 33.9 (CH_2), 31.9 (CH_2), 29.7–29.6 (CH_2), 29.4 (CH_2), 25.6 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HRESIMS m/z 632.2312 [M + Na]$^+$ (calcd for $C_{32}H_{42}Cl_2FNO_5Na$, 632.2316).

Preparation of 1e. Compound **1** (10 mg) was treated with tetrabromophthalic anhydride (15 mg) and 4-(dimethylamino)pyridine (10 mg) in pyridine (1.0 mL) at room temperature for 48 h. The reaction product was purified by preparative TLC (hexanes−EtOAc = 1:1) to give compound **1e** (9 mg, R_f 0.4): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (1H), 6.74 (1H), 6.04 (1H), 6.02 (1H), 5.18 (1H, m), 4.08 (1H), 2.32 (1H), 2.15 (2H), 2.00 (4H), 1.43–1.26 (26H), 0.878 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.6 (C), 169.7 (C), 166.6 (C), 151.9 (CH), 149.8 (CH), 138.0 (C), 135.3 (C), 133.4 (C), 130.6 (C), 129.9 (C), 128.5 (C), 126.9 (CH), 126.6 (CH), 106.7 (C), 79.5 (C), 71.4 (CH), 65.2 (CH), 39.4 (CH_2), 35.8 (CH_2), 34.3 (CH_2), 34.2 (CH_2), 31.9 (CH_2), 29.8–29.7 (CH_2), 29.4 (CH_2), 25.6 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HRESIMS m/z 904.9512 [M + Na]$^+$ (calcd for $C_{34}H_{42}Br_4O_7Na$, 904.9520).

Preparation of 1f. Compound **1** (10 mg) was treated with tetrachlorophthalic anhydride (15 mg) and 4-(dimethylamino)pyridine (10 mg) in pyridine (1.0 mL) at room temperature for 24 h. The reaction product was purified by preparative TLC (hexanes−EtOAc = 1:1) to give compound **1f** (7 mg, R_f 0.4): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.81 (1H), 6.78 (1H), 6.06 (1H), 6.04 (1H), 5.24 (1H), 4.09 (1H), 2.33 (1H), 2.20 (2H), 2.08 (4H), 1.42–1.25 (26H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.6 (C), 169.6 (C), 166.6 (C), 152.0 (CH), 149.9 (CH), 134.3 (C), 132.0 (C), 130.7 (C), 126.9 (CH), 126.6 (CH), 122.2 (C), 121.7 (C), 106.8 (C), 79.6 (C), 71.3 (CH), 65.3 (CH), 39.5 (CH_2), 35.8 (CH_2), 35.7 (CH_2), 34.3 (CH_2), 31.9 (CH_2), 29.8–29.7 (CH_2), 29.4 (CH_2), 25.6 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HRESIMS m/z 727.1562 [M + Na]$^+$ (calcd for $C_{34}H_{42}Cl_4O_7Na$, 727.1553).

Preparation of 1g. Compound **1** (20 mg) was treated with *p*-bromobenzoyl chloride (40 mg) and 4-(dimethylamino)pyridine (20 mg) in pyridine (1.0 mL) at room temperature for 24 h. The reaction mixture was purified using silica gel column chromatography (hexanes−EtOAc = 3:1) to afford 18 mg of **1g**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (2H), 7.47 (2H), 6.94 (1H), 6.67 (1H), 6.16 (1H), 6.04 (1H), 5.39 (1H), 4.23 (1H), 2.38 (1H), 2.26 (1H), 2.05 (2H), 2.04 (1H), 2.04 (1H), 1.91 (1H), 1.58 (1H), 1.47 (2H), 1.47 (1H), 1.41 (1H), 1.33–1.23 (20H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 186.1 (C), 165.9 (C), 152.2 (CH), 149.9 (CH), 132.0 (CH), 131.6 (CH), 130.1 (C), 128.4 (C), 127.3 (CH), 127.0 (CH), 107.7 (C), 79.5 (C), 68.1 (CH),

66.1 (CH), 39.8 (CH_2), 37.0 (CH_2), 36.3 (CH_2), 35.1 (CH_2), 34.5 (CH_2), 32.3 (CH_2), 30.2–30.0 (CH_2), 29.7 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 14.4 (CH_3); HRESIMS *m/z* 625.2335 $[M + Na]^+$ (calcd for $C_{33}H_{45}BrO_5Na$, 625.2328).

Preparation of 1h. Compound **1** (22 mg) was oxidized with pyridinium chlorochromate (40 mg) in CH_2Cl_2−pyridine (1:1, 2 mL) at room temperature for 24 h. The reaction mixture was purified using silica gel column chromatography (dicholoromethane) to yield 20 mg of **1h**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (1H), 6.75 (1H), 6.13 (1H), 6.11 (1H), 4.13 (1H), 2.77 (1H), 2.55 (1H), 2.42 (1H), 2.41 (1H), 2.40 (1H), 2.23 (1H), 2.11 (1H), 2.10 (1H), 1.66 (1H), 1.55 (1H), 1.38 (2H), 1.23–1.35 (20H), 0.88 (3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.5 (C), 185.2 (C), 50.3 (CH), 148.5 (CH), 127.5 (CH), 127.1 (CH), 109.5 (C), 79.7 (C), 69.6 (CH), 50.2 (CH_2), 47.2 (CH_2), 38.6 (CH_2), 36.1 (CH_2), 34.8 (CH_2), 31.8 (CH_2), 29.5–29.6 (CH_2), 29.3 (CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.1 (CH_3); HRESIMS *m/z* 439.2828 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_4Na$, 439.2819).

Preparation of 2a. Aculeatin B (**2**) (5.0 mg) was acetylated according to the same method described for **1a** to give aculeatin B 4-acetate (**2b**, 2.5 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, dd, J = 10.1, 3.0 Hz, H-13), 6.75 (1H, dd, J = 10.1, 3.0 Hz, H-9), 6.15 (1H, dd, J = 10.1, 2.0 Hz, H-12), 6.11 (1H, dd, J = 10.1, 2.0 Hz, H-10), 5.26 (1H, pentet, J = 3.0 Hz, H-4eq), 3.74 (1H, brt, J = 7.3 Hz, H-2ax), 2.63 (1H, dd, J = 12.9, 6.8 Hz, H-14a), 2.32 (1H, dt, J = 12.6, 7.6 Hz, H-15a), 2.09 (3H, s, $COCH_3$), 2.09 (1H, m, H-5a), 2.06 (1H, m, H-15b), 1.99 (1H, m, H-5b), 1.84 (1H, dd, J = 12.9, 8.3 Hz, H-14b), 1.73 (1H, brd, J = 14.1 Hz, H-3a), 1.61 (1H, m, H-16a), 1.54 (1H, m, H-3b), 1.48 (1H, m, H-17a), 1.47 (1H, m, H-16b), 1.32 (1H, m, H-17b), 1.33–1.23 (20H, m, H-18 to H-27), 0.88 (3H, t, J = 6.8 Hz, H-28); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.5 (C, C-11), 170.0 (C, *C*OO), 151.9 (CH, C-13), 148.8 (CH, C-9), 127.2 (CH, C-10), 127.2 (CH, C-12), 108.2 (C, C-6), 77.8 (C, C-8), 70.1 (CH, C-2), 68.4 (CH, C-4), 37.5 (CH_2, C-5), 35.7 (CH_2, C-16), 35.2 (CH_2, C-15), 34.8 (CH_2, C-14), 34.7 (CH_2, C-3), 31.9 (CH_2, C-26), 29.7–29.6 (CH_2, C-18 to C-24), 29.4 (CH_2, C-25), 25.8 (CH_2, C-17), 22.7 (CH_2, C-27), 21.4 (CH_3, OCO*C*H_3), 14.1 (CH_3, C-28); HRESIMS *m/z* 483.3083 $[M+ Na]^+$ (calcd for $C_{28}H_{44}O_5Na$, 483.3080).

Biological Evaluation. Chromatographic fractions were tested in the MCF-7 (human breast carcinoma) cell line during activity-guided fractionation. All isolates were assessed with the Lu1 (human lung carcinoma), LNCaP (hormone-dependent human prostate carcinoma), and MCF-7 cancer cell lines, using established protocols.[16,17] Aculeatin (**1**) was evaluated in the in vivo hollow fiber model, using Lu1, LNCaP, and MCF-7 cells, according to a procedure described in the literature.[18–21] Aculeatin A (**1**) was tested further in the in vivo P388 murine lymphocytic leukemia model, as described previously.[22,23] Finally, aculeatin A (**1**) was also evaluated in an in vivo human A2780 ovarian carcinoma murine xenograft model, performed according to a previously published protocol.[24,25]

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Supporting Information Available: Data for in vivo hollow fiber evaluation of aculeatin A (**1**) against Lu1 (human lung cancer) and LNCaP (human prostate cancer) cells and spectroscopic data for compounds **3**−**7**. This information is provided free of charge via the Internet at http://pubs.acs.org.

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