Sesquiterpene Benzoxazoles and Sesquiterpene Quinones from the Marine Sponge *Dactylospongia elegans*

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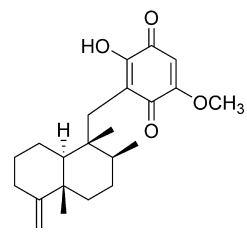
A new sesquiterpene benzoxazole, nakijinol B (**3**), its acetylated derivative, nakijinol B diacetate (**6**), and two new sesquiterpene quinones, smenospongines B (**4**) and C (**5**), were isolated from the methanol extract of the marine sponge *Dactylospongia elegans.* Also isolated were the known compounds dactyloquinone B and a 1:1 mixture of ilimaquinone and 5-*epi*-ilimaquinone. Their structures were determined on the basis of spectroscopic analyses and comparison with literature data. The isolated compounds were assessed for their cytotoxicity against a panel of human tumor cell lines (SF-268, H460, MCF-7, and HT-29) and a normal mammalian cell line (CHO-K1). All compounds were found to have activities in the range 1.8−46 μM and lacked selectivity for tumor versus normal cell lines.

Sponges of the genus *Dactylospongia*,[1−10] in particular *D. elegans*,[1−7] have been widely investigated for their biologically active compounds. Aside from a lone macrolide,[8] the majority of the reported metabolites are sesquiterpene quinones/quinols,[1,2,4−7,9] whose occurrence has been noted in a variety of other genera of marine sponges including *Fenestraspongia*,[11] *Petrosaspongia*,[12] *Hyrtios*,[13] *Spongia*,[14] *Dysidea*,[15] and *Smenospongia*.[16] Other terpenoids isolated from *Dactylospongia* spp. include aromatic sesquiterpenes,[3,4,7] sesquiterpene cyclopentanones,[4,9] γ-lactones,[4,5] and sesterterpene lactones.[10] Several of these reports also describe associated cytotoxic activity of isolated sesquiterpene quinones/quinols.[1,3,5,7,13,14,16]

As part of our continued interest in the search for anticancer agents, EtOH extracts of marine flora and invertebrate fauna were tested for activity against the NCI-60 cancer cell line panel (COMPARE http://itbwork.nci.nih.gov/docs/compare/compare.html). From this screening, an extract of the sponge *D. elegans* was identified as having strong cytotoxic activity in addition to a unique COMPARE analysis profile. Subsequent bioassay-guided fraction of a large-scale MeOH extract of *D. elegans* led to the isolation of the known sesquiterpene quinones ilimaquinone (**1**)[17,18] and 5-*epi*-ilimaquinone[11] as a 1:1 mixture, as well as dactyloquinone B (**2**).[2] Also isolated were a new sesquiterpene benzoxazole, nakijinol B (**3**), and the new sesquiterpene quinones smenospongines B (**4**) and C (**5**). Subsequent acetylation of the MeOH-insoluble material yielded the diacetate derivative of **3**, nakijinol B diacetate (**6**). Discussed below in detail are the isolation, the structure elucidation, and the determination of the relative configurations for **3**−**6**, as well as the biological activities of **1**−**6** against a panel of human tumor cell lines (SF-268, H460, MCF-7, and HT-29) and a normal mammalian cell line (CHO-K1).



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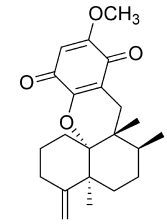

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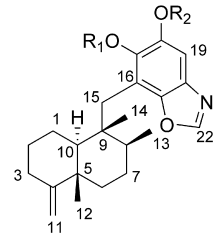

3 $R_1 = R_2 = H$
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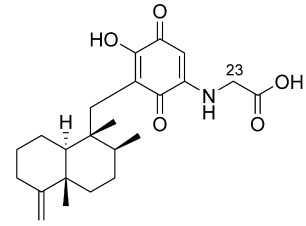

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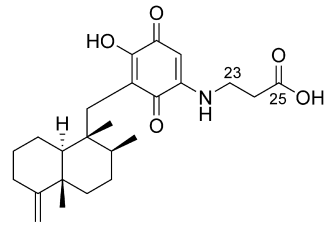

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Results and Discussion

Nakijinol B (**3**) was isolated as a colorless solid with a molecular weight ([M + Na]$^+$ *m/z* 378.2050) consistent with the molecular formula $C_{22}H_{29}NO_3$ and corresponding to nine double-bond equivalents. The IR spectrum of **3** indicated absorptions due to the presence of aromatic nitrogen (ν_{max} 1541 cm^{-1}) and hydroxy (ν_{max} 3408 cm^{-1}) moieties. Analysis of the ^{1}H and ^{13}C NMR data of **3** (Table 1) readily identified resonances consistent with an exomethylene (δ_C 161.8; 103.4; δ_H 4.35, s; 4.32, s), a pentasubstituted aromatic ring (δ_C 146.7; 146.7; 145.2; 131.7; 111.0; 102.6; δ_H 6.96, s), and an N=C bond (δ_C 153.2; δ_H 8.20, s; IR ν_{max} 1541 cm^{-1}). This accounted for six of nine double-bond equivalents and, in the absence of any other sp or sp^2 carbons, indicated that **3** had three additional rings. Also evident in the NMR spectra were resonances consistent with one secondary methyl (δ_C 19.2; δ_H 1.08, d, J = 6.8

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Table 1. NMR Spectroscopic Data (CD_3OD) for Nakijinol B (**3**)

no.	δ_C, mult.[a]	δ_H (J in Hz)[b]	COSY	gHMBC[c]
1	24.8, CH_2	2.37, m	H_b-1, H_a-2, H-10	2
		1.55, m	H_a-1, H-10	
2	30.2, CH_2	1.87, m	H_b-2, H_a-3	
		1.28, m	H_a-1, H_a-2, H_2-3	
3	34.4, CH_2	2.32, m	H_b-2, H_b-3	2, 4, 11
		2.03, m	H_a-2, H_a-3	5
4	161.8, C			
5	41.8, C			
6	38.3, CH_2	1.45, m		8
7	29.5, CH_2	1.41, m		
8	38.7, CH	1.41, m	H_3-13	
9	44.2, C			
10	51.1, CH	0.94, m	H_2-1	5, 9, 12, 14
11	103.4, CH_2	4.35, s		3, 4, 5
		4.32, s		3, 4, 5
12	20.7, CH_3	1.07, s		4, 5, 6, 10
13	19.2, CH_3	1.08, d (6.8)	H-8	7, 8, 9
14	18.3, CH_3	0.95, s		8, 9, 10, 15
15	35.8, CH_2	2.94, d (13.9)	H_b-15	8, 9, 10, 14, 16, 17, 21
		2.86, d (13.9)	H_a-15	8, 9, 10, 14, 16, 17, 21
16	111.0, C			
17	146.7, C			
18	145.2, C			
19	102.6, CH	6.96, s		17, 18, 20, 21
20	131.7, C			
21	146.7, C			
22	153.2, CH	8.20, s		20, 21

[a] 75 MHz. [b] 300 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbons.

Hz), two tertiary methyls (δ_C 20.7; 18.3; δ_H 1.07, s; 0.95, s), six methylenes, two methines, and two quaternary sp^3 carbons.

Analysis of the two-dimensional NMR data for **3** (Table 1) readily identified a *trans*-4,9-friedodrimane ring system, identical to that found in ilimaquinone (**1**).[17,18] Observed gHMBC correlations from H_2-15 (δ_H 2.94, d, J = 13.9 Hz; 2.86, d, J = 13.9 Hz) to C-16 (δ_C 111.0), C-17 (δ_C 146.7), and C-21 (δ_C 146.7) and from H-19 (δ_H 6.96, s) to C-17, C-18, C-20 (δ_C 131.7), and C-21 established the connectivity between the pentasubstituted aryl moiety and the *trans*-4,9-friedodrimane ring system. The observation of gHMBC correlations from H-22 (δ_H 8.20, s) to C-20 (δ_C 131.7) and C-21 (δ_C 146.7), in addition to the ^{13}C chemical shifts of C-20, C-21, and C-22 (δ_C 153.2), were indicative of **3** containing a benzoxazole moiety.[19] These observations accounted for all atoms aside from two hydroxy moieties. Only two points of attachment were available: C-17 (δc 146.7) and C-18 (δc 145.2). These resonances were consistent with aromatic hydroxy groups, hence establishing the structure of **3** as shown. Comparison of the NMR data for **3** with that of the known compound nakijinol[20] revealed the two to be very similar, the only difference being that **3** contains an exo rather than an endo double bond in the *trans*-4,9-friedodrimane ring system.

Smenospongine B (**4**) was isolated as an amorphous, red solid with a molecular weight ($[M + Na]^+$ m/z 424.2104) consistent with the molecular formula $C_{23}H_{31}NO_4$ and corresponding to nine double-bond equivalents. The NMR data for **4** (Table 2) were almost identical to that for **1**,[17,18] with the only difference being the resonance for C-20 (**4**: δ_C 150.9; **1**: δ_C 161.8). Observed gHMBC correlations from H_2-23 to C-20 and C-24 (δ_C 171.7) and the ^{13}C chemical shift of C-23 (δ_C 44.7) established a 2-aminoacetic acid moiety at C-20. A spectrum acquired in DMSO-d_6 confirmed this moiety, where gHMBC correlations from H-22 (δ_H 7.56, t, J = 5.8 Hz) to C-19 (δ_C 92.9) and C-21 (δ_C 181.8) were observed in addition to a COSY correlation between H-22 (δ_H 7.56, t, J = 5.8 Hz) and H_2-23 (δ_H 3.88, d, J = 5.8 Hz). The relative configuration of **4** was established to be the same as those for **1** and **3** by comparison of the ^{13}C NMR data. Hence the gross structure and relative configuration of **4** were elucidated as shown.

Smenospongine C (**5**) was isolated as an amorphous, red solid with a molecular weight ($[M + Na]^+$ m/z 438.2264) consistent with the molecular formula $C_{24}H_{33}NO_4$ and corresponding to nine double-

Table 2. NMR Spectroscopic Data for Smenospongine B (**4**)

no.	δ_C, mult.[a,b]	δ_H (J in Hz)[a,c]	COSY[a]	gHMBC[a,d]	δ_C, mult.[b,e]	δ_H (J in Hz)[c,e]
1	24.2, CH_2	2.19, br d (12.7)	H_b-1, H_2-2, H-10	2, 15	22.7, CH_2	2.09, m
		1.48, m	H_a-1, H_a-2, H-10			1.35, m
2	29.7, CH_2	1.84, dd (12.7, 3.0)	H_b-1, H_b-2, H_2-3		28.0, CH_2	1.74, m
		1.23, m	H_a-1, H_a-2, H_b-3			1.14, m
3	34.0, CH_2	2.35, ddd (13.8, 5.2, 3.0)	H_a-2, H_b-3, H_2-11	4	32.1, CH_2	2.23, m
		2.04, dd (13.8, 5.2)	H_a-2, H_a-3	1, 2, 4, 11		1.99, m
4	161.4, C				159.3, C	
5	41.3, C				39.8, C	
6	37.8, CH_2	1.51, m	H_b-6, H_a-7	8, 10	36.4, CH_2	1.43, m
		1.41, m	H_a-6	5, 7, 8, 12		1.27, m
7	28.8, CH_2	1.40, m	H_2-6	6, 8, 9	27.6, CH_2	1.32, m
						1.11, m
8	38.9, CH	1.25, m	H_a-7, H_3-13	7, 13	37.3, CH	1.17, m
9	43.7, C				42.2, C	
10	50.9, CH	0.85, m	H_2-1	1, 8, 9, 12, 15	49.3, CH	0.74, m
11	103.1, CH_2	4.40, br s	H_a-3, H_2-11	3, 4, 5	102.9, CH_2	4.40, s
						4.37, s
12	20.9, CH_3	1.05, s		4, 5, 6, 10	20.2, CH_3	0.97, s
13	18.5, CH_3	0.98, d (6.3)	H-8	7, 8, 9	18.1, CH_3	0.92, d (6.2)
14	17.4, CH_3	0.84, s		8, 9, 10, 15	17.3, CH_3	0.76, s
15	33.1, CH_2	2.50, d (13.8)	H_b-15	8, 9, 10, 14, 16, 17, 21	32.0, CH_2	2.36, d (13.6)
		2.40, d (13.8)	H_a-15	8, 9, 10, 14, 16, 17, 21		2.27, d (13.6)
16	115.4, C				113.2, C	
17	159.4, C				159.7, C	
18	182.2, C				180.4, C	
19	93.6, CH	5.27, s		17, 18, 21	92.9, CH	5.19, s
20	150.9, C				149.6, C	
21	183.8, C				181.8, C	
22						7.56, t (5.8)
23	44.7, CH_2	3.94, br s		20, 24	42.1, CH_2	3.88, d (5.8)
24	171.7, C				169.8, C	

[a] CD_3OD. [b] 75 MHz. [c] 300 MHz. [d] HMBC correlations are from proton(s) stated to the indicated carbons. [e] DMSO-d_6.

Table 3. GI_{50} (μM) Data for Compounds **1**−**6** against SF-268, MCF-7, H460, HT-29, and CHO-K1 Cells

compound	SF-268[a]	MCF-7[b]	H460[c]	HT-29[d]	CHO-K1[e]
1	2.7	3.9	1.8	5.4	2.0
2	32	41	30	46	43
3	24	35	24	21	11
4	9.7	10	6	6.0	3.0
5	20	31	14	28	18
6	9	19	6.8	15	5.2

[a] SF-268: Central nervous system-glioblastoma cells. [b] MCF-7: Breast-pleural effusion adenocarcinoma cells. [c] H460: Lung-large cell carcinoma cells. [d] HT-29: Colon-recto-sigmoid colon adenocarcinoma cells. [e] CHO-K1: Subclone of Chinese hamster ovary cells.

bond equivalents. Comparison of the NMR data for **5** with those for **4** revealed almost identical data, with the only differences being the resonances for C-23 (**4**: δ_C 44.7; δ_H 3.94, br s; **5**: δ_C 39.9; δ_H 3.45, t, $J = 6.8$ Hz) and the presence of an additional methylene (C-24: δ_C 34.5; δ_H 2.59, t, $J = 6.8$ Hz). The observation of a COSY correlation from H_2-23 to H_2-24 and gHMBC correlations from H_2-24 to C-23 (δ_C 39.9) and C-25 (δ_C 176.0) established a 3-aminopropionic acid moiety for **5**, as opposed to the 2-aminoacetic acid moiety in **4**. In all other respects **4** and **5** were identical. Hence the gross structure and relative configuration for **5** are as shown.

Acetylation of the MeOH-insoluble material from the original extraction and subsequent purification afforded two active fractions. Nakijinol B diacetate (**6**) was isolated as a colorless oil, with a molecular weight ($[M + Na]^+$ *m/z* 462.2250) consistent with the molecular formula $C_{26}H_{33}NO_5$ and corresponding to 11 double-bond equivalents. Comparison of the NMR data of **6** with those of **3** showed that the two data sets were virtually identical. All differences were accounted for by the presence of two acetate moieties (δ_C 168.1, 167.9, 2 × 20.1; δ_H 2.34, s; 2.30, s) in **6**, as opposed to the two hydroxy moieties found in **3**. The relative configuration of **6** was identical to **3**, indicative of **6** being formed through the acetylation of residual **3** in the MeOH-insoluble material.

An additional four acetylated stereoisomers of **4** were also isolated as a mixture. Further purification, however, was not possible due to decomposition (tentative structure elucidation of the four compounds is available in the Supporting Information).

The biological activities of **1**−**6** were established against a panel of human tumor cell lines, as well as a normal mammalian cell line (Table 3). The compounds were found to have cytotoxic activities in the range 1.8−46 μM and appeared to lack selectivity for tumor versus normal cell lines. The 1:1 mixture of ilimaquinone (**1**) and 5-*epi*-ilimaquinone was found to be the most cytotoxic, with GI_{50} values ranging from 1.8 to 5.4 μM, which is consistent with previous findings.[1,16] The presence of two bulky acetate moieties resulted in an approximate 2-fold increase in the activity of **6** compared to the diol (**3**). One possible explanation for this increase in activity is that the acetate groups may contribute to greater bioavailability through enhanced membrane permeation after which metabolism, possibly hydrolysis by esterases, releases the active compounds intracellularly.[21] For **4** and **5**, the additional methylene in the nitrogen-substituted side chain had the effect of reducing observed activity by a factor of 2. This is the first report of cytotoxic activity for **2**.

Experimental Section

General Experimental Procedures. General experimental details are as previously reported.[22,23]

Sponge Material. The sponge *Dactylospongia elegans* (order Dictyoceratida, family Thorectidae) was collected from Pugh Shoal, northeast of Truant Island, NT, in November 1990 (11°36′6″ S, 136°53′4″ E) and frozen. Collection was conducted under the Aboriginal Lands Council permit number DHA/644-651 GAL/134-141. A voucher specimen (accession number G311929) has been lodged with the Queensland Museum.

Extraction and Isolation. Freeze-dried sponge material (33 g dried weight) was extracted with MeOH (3 × 1 L), concentrated *in vacuo*, and subject to reversed-phase C18 vacuum liquid chromatography (0%, 20%, 50%, 70%, 90%, 100% MeOH in H_2O, and 1:1 CH_2Cl_2/MeOH). The bioactive fractions (90% and 100% MeOH) were combined, preadsorbed onto C_{18} silica gel, and subjected to C_{18} preparative HPLC (9.5 mL/min, gradient elution from 3:7 H_2O/MeCN (+0.1% formic acid) to 100% MeCN (+0.1% formic acid) over 20 min, then 20 min with 100% MeCN (+0.1% formic acid) through a 250 × 21 mm, 5 μm Phenomenex Luna C18 column) to yield a 1:1 mixture of ilimaquinone (**1**)[17,18] and 5-*epi*-ilimaquinone[11] (218 mg, 0.66%), as well as three other fractions containing active minor compounds. Each of these fractions was further purified using identical HPLC conditions (4 mL/min, gradient elution from 3:7 H_2O/MeCN/0.1% formic acid to 100% MeCN/0.1% formic acid over 10 min, through a 150 × 10 mm, 5 μm Phenomenex phenyl hexyl column) to yield the known compound dactyloquinone B[2] (**2**, 4.7 mg, 0.018%) and the new compounds nakijinol B (**3**, 0.8 mg, 0.002%), smenospongine B (**4**, 11.7 mg, 0.035%), and smenospongine C (**5**, 1.4 mg, 0.004%).

Mixture of ilimaquinone (1) and 5-*epi*-ilimaquinone (1:1): spectroscopic data identical in all aspects to those previously reported.[11,17,18]

Dactyloquinone B (2): spectroscopic data identical in all aspects to those previously reported.[2]

Nakijinol B (3): colorless solid; $[\alpha]_D$ −6.7 (*c* 0.075, MeOH); UV (PDA, CH_3CN/H_2O) λ_{max} 236, 297, 323 (sh) nm; IR (neat) ν_{max} 3408, 2927, 1541 cm^{-1}; ^1H (300 MHz) and ^{13}C (75 MHz) NMR (CD_3OD), see Table 1; HRESIMS *m/z* 378.2050 $[M + Na]^+$ (calcd for $C_{22}H_{29}NO_3Na$, 378.2040, Δ 1.0 mmu).

Smenospongine B (4): amorphous, red solid; $[\alpha]_D$ +94.4 (*c* 0.018, MeOH); UV (PDA, CH_3CN/H_2O) λ_{max} 218, 311, 494 nm; IR (neat) ν_{max} 3598, 2936, 2064, 1657 cm^{-1}; ^1H (300 MHz) and ^{13}C (75 MHz) NMR (CD_3OD), see Table 2; HRESIMS *m/z* 424.2104 $[M + Na]^+$ (calcd for $C_{23}H_{31}NO_5Na$, 424.2094, Δ 1.0 mmu).

Smenospongine C (5): amorphous, red solid; $[\alpha]_D$ +13 (*c* 0.06, MeOH); UV (PDA, CH_3CN/H_2O) λ_{max} 233, 314, 494 nm; IR (neat) ν_{max} 3410, 2930, 1686, 1632, 1567 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.38 (1H, s, H-19), 4.40 (2H, s, H_2-11), 3.45 (2H, t, $J = 6.8$ Hz, H_2-23), 2.59 (2H, t, $J = 6.8$ Hz, H_2-24), 2.47 (1H, d, $J = 13.6$ Hz, H_a-15), 2.38 (1H, d, $J = 13.6$ Hz, H_b-15), 2.32 (1H, m, H_a-3), 2.16 (1H, m, H_a-1), 2.04 (1H, m, H_b-3), 1.80 (1H, m, H_a-2), 1.48 (1H, m, H_a-6), 1.43 (1H, m, H_b-1), 1.41 (1H, m, H_a-7), 1.36 (1H, m, H_b-6), 1.35 (1H, m, H_b-7), 1.29 (1H, m, H_b-2), 1.21 (1H, m, H-8), 1.04 (3H, s, H_3-12), 0.97 (3H, d, $J = 6.4$ Hz, H_3-13), 0.83 (3H, s, H_3-14), 0.81 (1H, m, H-10); ^{13}C NMR (CD_3OD, 75 MHz) δ 183.4 (C, C-21), 179.6 (C, C-18), 176.0 (C, C-25), 162.9 (C, C-4), 161.1 (C, C-17), 152.0 (C, C-20), 115.5 (C, C-16), 103.3 (CH_2, C-11), 92.6 (CH, C-19), 51.1 (CH, C-10), 44.0 (C, C-9), 41.8 (C, C-5), 39.9 (CH_2, C-23), 39.0 (CH, C-8), 38.2 (CH_2, C-6), 34.5 (CH_2, C-24), 34.2 (CH_2, C-3), 33.4 (CH_2, C-15), 30.0 (CH_2, C-2), 29.4 (CH_2, C-7), 24.3 (CH_2, C-1), 21.4 (CH_3, C-12), 18.7 (CH_3, C-13), 18.1 (CH_3, C-14); HRESIMS *m/z* 438.2264 $[M + Na]^+$ (calcd for $C_{24}H_{33}NO_5Na$, 438.2251, Δ 1.3 mmu).

Acetylation of MeOH-Insoluble Material. A solution of MeOH-insoluble material (120 mg) in pyridine (0.5 mL) was treated with $(CH_3CO)_2O$ (0.5 mL) and stirred at room temperature for 12 h. The reaction material was concentrated *in vacuo*, then subjected to sequential reversed-phase HPLC separations (A: H_2O/MeCN + 0.1% formic acid (3:7) to 100% MeCN + 0.1% formic acid over 10 min at 4 mL/min and held for an additional 10 min on a 150 × 10 mm, 5 μm Phenomenex Luna C18 column; B: H_2O/MeOH with 0.1% formic acid (3:7) to 100% MeOH with 0.1% formic acid for 10 min at 4 mL/min and held for an additional 5 min on a 150 × 10 mm, 5 μm Phenomenex Luna phenyl hexyl column) to yield more dactyloquinone B (**2**, 1.2 mg, 0.004%) and nakijinol B diacetate (**6**, 2.3 mg, 0.007%). An additional four acetylated stereoisomers of **4** were also isolated as a mixture. Further purification was not possible due to decomposition (tentative structure elucidation of the four compounds is available in the Supporting Information).

Nakijinol B diacetate (6): colorless oil; $[\alpha]_D$ −170 (*c* 0.003, $CHCl_3$); UV (PDA, CH_3CN/H_2O) λ_{max} 233, 278, 284, 300 (sh) nm; IR (neat) ν_{max} 3488, 2927, 1775, 1630, 1458 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.07 (1H, s, H-22), 7.54 (1H, s, H-19), 4.42 (1H, d, $J = 1.6$ Hz, H_a-11), 4.38 (1H, d, $J = 1.6$ Hz, H_b-11), 2.83 (1H, d, $J = 14.2$ Hz, H_a-15), 2.76 (1H, d, $J = 14.2$ Hz, H_b-15), 2.35 (1H, m, H_a-3), 2.34 (s, a-OCO$\underline{CH_3}$), 2.30 (s, b-OCO$\underline{CH_3}$), 2.08 (1H, m, H_b-3), 1.92 (1H, m,

H_a-2), 1.58 (1H, m, H_a-1), 1.49 (1H, m, H_a-6), 1.45 (1H, m, H_b-1), 1.43 (1H, m, H-8), 1.42 (2H, m, H_2-7), 1.28 (1H, m, H_b-6), 1.26 (1H, m, H_b-2), 1.07 (3H, s, H_3-12), 0.97 (3H, d, $J = 5.6$ Hz, H_3-13), 0.94 (3H, s, H_3-14), 0.92 (1H, m, H-10); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 168.1 (C, a-O$\underline{C}$OCH$_3$), 167.9 (C, b-O$\underline{C}$OCH$_3$), 159.1 (C, C-4), 152.7 (CH, C-22), 147.9 (C, C-21), 140.4 (C, C-17), 137.4 (C, C-18), 136.6 (C, C-20), 118.3 (C, C-16), 111.9 (CH, C-19), 102.2 (CH_2, C-11), 50.1 (CH, C-10), 43.0 (C, C-9), 40.3 (C, C-5), 37.6 (CH, C-8), 36.2 (CH_2, C-6), 35.6 (CH_2, C-15), 32.6 (CH_2, C-3), 28.3 (CH_2, C-2), 27.7 (CH_2, C-7), 23.1 (CH_2, C-1), 20.1 (2 × CH_3, −OCO$\underline{C}$H$_3$), 19.9 (CH_3, C-12), 18.1 (CH_3, C-13), 16.8 (CH_3, C-14); HRESIMS *m*/*z* 462.2250 [M + Na]$^+$ (calcd for $C_{26}H_{33}NO_5Na$, 462.2251, Δ 0.1 mmu).

Bioassay. Briefly, MCF-7, SF-268, and H460 cells were grown in RPMI 1640 medium with L-glutamine supplemented with 5% fetal bovine serum and maintained in a humidified incubator at 37 °C with 5% CO_2. Conversely, CHO-K1 and HT-29 cells were grown in minimum essential medium (MEM) with Earle's salts, L-glutamine, nonessential amino acids, and sodium pyruvate. Samples to be tested were solubilized in DMSO, serially diluted in medium, and added to 96-well microtiter plates (5000 cells/well in 100 μL of medium) so that the final doses ranged from 250 to 3 μg/mL. Pure compounds were tested at 10 concentrations within the dose range 66.7−0.01 μg/mL. Total cellular protein, as an indicator of cell number, was measured (Wallac Victor Plate reader, 490 nm) at 0 and 48 h after sample addition using the sulforhodamine B (SRB) assay.[24] Inhibition of growth by 50% (GI_{50}) was determined by comparing the sample-treated values to those of vehicle-only control and time 0 readings.

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Supporting Information Available: NMR spectra for all isolated compounds and tentative structure elucidation of acetylated derivatives of **4** are available free of charge via the Internet at http://pubs.acs.org.

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