Candidaspongiolides, Distinctive Analogues of Tedanolide from Sponges of the Genus *Candidaspongia*

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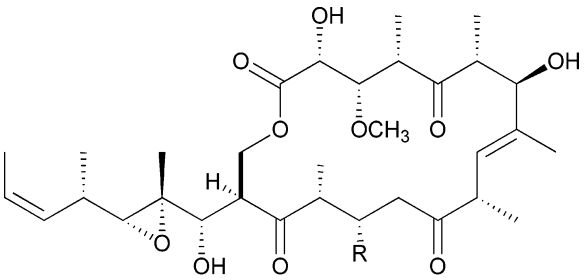
Fractionation of cytotoxic extracts of specimens of a newly described sponge genus, *Candidaspongia*, has yielded the candidaspongiolides (**3**), a complex mixture of acyl esters of a macrolide related to tedanolide. The general structure of the candidaspongiolides was determined by analyses of various 2D NMR and MS data sets. The acyl ester components were identified by GC-MS analysis of the derived fatty acid methyl esters. The mixture could be selectively converted to the deacylated macrolide core (**4**) by enzymolysis with immobilized porcine lipase, with the structure of the candidaspongiolide core then secured by NMR and MS analysis. The candidaspongiolide mixture was potently cytotoxic, exhibiting a mean panel 50% growth inhibition (GI_{50}) of 14 ng/mL in the National Cancer Institute's 60-cell-line *in vitro* antitumor screen.

Among the first natural products extract leads selected for study on the basis of data from the U.S. National Cancer Institute's human disease-oriented, 60-cell-line primary antitumor screen[1−3] were the aqueous and organic extracts of a then undescribed new sponge genus (collection Q66C0330). Both extracts exhibited significant cytotoxicity in the primary antitumor screen, as well as selective or differential activity toward melanoma and central nervous system tumor cell lines, with mean panel LC_{50} values of less than 1 μg/mL. This relatively uncommon sponge was already the object of scrutiny at the Australian Institute of Marine Science (AIMS), as part of a chemotaxonomic study of folliose Dictyoceratid sponges of the Great Barrier Reef[4] and for significant cytotoxicity in a P388 murine tumor cell line assay. Paucity of the sponge material and concomitant conservation considerations, together with a common focus on the antitumor potential of its secondary metabolites, prompted a collaborative effort to isolate and characterize the cytotoxic constituents. Ultimately, the acquisition of two collections of the same genus from Papua New Guinea, containing the same mixture of compounds, permitted completion of the isolation, characterization, and biological testing of the candidaspongiolides, a complex series of novel analogues of tedanolide (**1**).[5]

Results and Discussion

At AIMS, the dichloromethane extract of lyophilized sponge was fractionated by flash chromatography on silica gel with hexane−EtOAc mixtures. The cytotoxic fractions were further separated by normal-phase HPLC (silica, hexane−EtOAc, 3:2) to give a cytotoxic, semicrystalline fraction. That product initially appeared to be a pure compound related to tedanolide (**1**) and 13-deoxytedanolide (**2**), previously isolated from *Tedania ignis*[5] and *Mycale adhaerens*,[6] respectively, differing primarily by the presence of a long-chain fatty acid ester moiety. Subsequent reversed-phase HPLC (C_{18}, MeOH−H_2O, 9:1) analysis suggested that the material was, in fact, a complex mixture of compounds comprised of the same core macrolide esterified by a series of fatty acids.



1 R = OH
2 R = H

At the NCI, a few of the more abundant members of the mixture could be isolated by painstaking, repeated HPLC, but the yields and recovery were poor. Given the concerns about available sponge biomass at the time, we instead opted for a three-step approach to identifying the candidaspongiolides: (1) to determine the gross structure of the candidaspongiolide esters using the purified mixture; (2) to identify the fatty acids present as esters and estimate their relative abundance; and (3) to attempt to convert the candidaspongiolide ester mixture to a single, simple candidaspongiolide core molecule.

Gross Structure. The close structural relationship of the candidaspongiolide mixture (**3**) to tedanolide quickly became apparent from analysis of COSY and TOCSY spectra, which indicated that the major modifications in structure (versus tedanolide) were centered in the C-11 to C-15 segment of the macrolide skeleton. The COSY spectrum contained correlations for a methylene AB pair (δ 2.47 and 2.66, H-12) coupled to a methine proton at δ 4.40 (H-13), which was, in turn, coupled to a hydroxyl proton at δ 3.45. The COSY spectrum also contained correlations for an isolated methylene (δ 4.44 and 4.19, H-28a, b). Unaccounted for was the last of four exchangeable OH protons (δ 3.12). HMBC experiments revealed correlations from the H-12, H-13, and OH-13 protons to a quaternary carbon at δ 81.6, consistent with its placement at C-14. On the basis of the chemical shift of this carbon,

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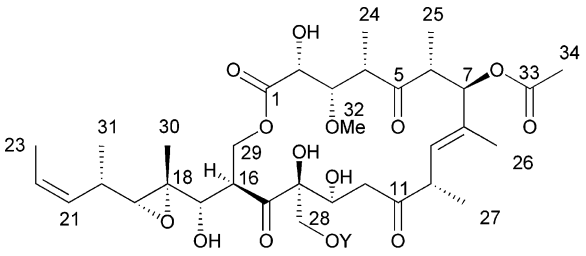
Table 1. NMR Spectroscopic Assignments (500 MHz, $CDCl_3$) for Candidaspongiolide Mixture **3**

position	δ_C mult.	δ_H mult. (*J* in Hz)	HMBC[a]	NOE[b]
1	171.3 qC			
2	70.7 CH	3.96 dd (7.3, 1.0)	1	3, 4, 26
		OH 2.85 d (7.3)	1	
3	83.4 CH	3.64 dd (7.8, 1.0)	4, 5, 32	2, 24, 32
4	47.8 CH	3.12 m	5, 32	2, 6, 24, 26
5	214.6 qC			
6	48.5 CH	3.18 dq (10.7, 7.3)	5, 7, 8, 25	
7	80.1 CH	5.39 d (10.7)	6, 9, 25, 26, 33	9, 26, 34
8	131.6 qC			
9	132.0 CH	5.60 br d (9.3)	7, 11, 26, 27	7, 10, 27
10	46.2 CH	3.38 dq (9.3, 6.8)	11, 27	
11	211.5 CH			
12	42.6 CH_2	a 2.66 dd (16.1, 9.8)	11, 13, 14	2, 10, 12b
		b 2.47 br dd (16.1, 2.4)	11, 13	10, 12a, 13, 28a/b
13	68.9 CH	4.40 m	14, 15	12b, 16
		OH 3.45 d (4.9)	12, 13	
14	81.6 qC			
		OH 3.31 br s	15	
15	210.8 qC			
16	46.9 CH	4.02 dt (10.9, 3.7)	15, 17, 29	
17	77.8 qC	3.12 m	16, 18, 19	19, 29b
		OH 2.77 br d (2.9)	16, 17	
18	62.7 qC			
19	67.1 CH	2.56 d (9.3)	20	17, 21, 29b
20	31.1 CH	2.44 m	19, 21, 22	21, 23, 30
21	129.7 CH	5.23 dt (10.7, 1.5)	19	
22	125.5 CH	5.48 dq (10.7, 6.8)	21, 23	21, 23
23	13.5 CH_3	1.59 dd (6.8, 1.5)	20, 21, 22	20, 22, 30
24	14.6 CH_3	1.18 d (6.8)	3, 4, 5	3, 32
25	14.7 CH_3	1.13 d (7.3)	5, 6, 7, 8	6, 7
26	10.8 CH_3	1.54 br d (1.0)	7, 8, 9	2, 10
27	16.3 CH_3	1.07 d (6.8)	9, 10, 12	7, 10, 26
28	63.5 CH_2	a 4.44 d (11.7)	13, 15	12b
		b 4.19 d (11.7)	13, 15, 35	13
29	63.2 CH_2	a 4.10 dd (10.9, 10.2)	1, 15, 16	21, 29b, 30
		b 4.17 dd (9.8, 3.7)	1, 15, 16	12b
30	11.1 CH_3	1.38 s	17, 18, 19	17, 20, 29a/b
31	18.6 CH_3	1.11 d (6.4)	19, 20, 21	19, 20, 21
32	60.3 CH_3	3.28 s	3	3, 24
33	169.5 qC			
34	21.6 CH_3	2.01 s	33	7
35	173.5 qC			
36	34.2 CH_2	2.24 t (7.3)		
37	29 br CH_2	1.53 br	35	

[a] HMBC correlations, optimized at 8.3 and 5 Hz, are from protons to the indicated carbons. [b] NOE spectra recorded in MeOH-d_4.

the remaining OH and the isolated, deshielded methylene (*vide supra*) were placed there as its other substituents. This was supported by correlations from the OH proton (δ 3.31) to the neighboring ketone carbonyl at δ 210.8 (C-15). Correlations from the methylene pair at δ 4.19 and 4.44 to both C-13 and C-15 confirmed placement of these protons on C-28. Further correlations from H-28b to the ester carbonyl at δ 173.5 revealed this to be the point of attachment for the fatty acid ester, as suggested by the ^{1}H NMR chemical shifts for H-28. Other correlations present in the HMBC spectra supported the proposed structure for the mixture and are summarized in Table 1. The remaining structural elements were essentially unchanged from tedanolide, except for C-7, which was acetylated in the candidaspongiolides.

The presence of a fatty acid ester substituent on the macrolide was indicated in the ^{1}H NMR spectrum by a two-proton triplet at δ 2.24 (methylene α to carbonyl) coupled (COSY spectrum) to the very intense methylene envelope centered at δ 1.23. Further coupling from this envelope to several overlapped signals was observed in the terminal methyl group region (δ 0.80−0.95). HMQC experiments indicated that the broad methylene envelope signal was associated with multiple carbon signals between 29 and 30 ppm. The α-methylene signal (δ 2.24) was correlated to a carbon signal at δ 34.2 (HMQC) and adjacent to the ester carbonyl at δ 173.5 (HMBC). The upfield section of the ^{13}C NMR spectrum showed a diversity of chemical shifts for methyl, methylene, and methine carbons; these data, together with DEPT NMR data, led



3 Y =  R = $C_{13}H_{27}$; $C_{14}H_{29}$; $C_{15}H_{31}$; $C_{16}H_{33}$; $C_{17}H_{35}$; $C_{17}H_{33}$

4 Y = H

to the construction of fragments corresponding to the normal, *iso*, and *anteiso* isomers of the acyl esters.

Coupling constant analysis of the macrolide suggested that the relative stereochemistry was identical to that described for tedanolide[5] and 13-deoxytedanolide.[6] Difference NOE spectroscopy experiments were also consistent with this conclusion. Because of the functional group changes, the C-11−C-14 segment of the core provided the principal challenge. The coupling constants for H_2-12 [H-12a, dd, J = 16.1, 9.8 Hz; H-12b, dd, J = 16.1, 2.4 Hz] and H-13 [m, J = 9.8, 4.9, 2.4 Hz, inferred from H-12a/b, OH-13] were very similar to those observed for tedanolide [Table S1, Supporting

Table 2. FAME Composition of Candidaspongiolides (**3**)

fatty acid	composition (%)[a]
n-14:0	4.0
i-15:0	6.7
a-15:0	trace
n-15:0	2.8
i-16:0	15.8
a-16:0	5.5
n-16:0	21.3
i-17:0	8.3
a-17:0	9.4
n-17:0	2.7
n-18:0	8.8
n-18:1 (Δ^9)	4.7

[a] Approximately 10% of FAME mixture is minor unidentified compounds.

Information (H-12a, dd, J = 16.9, 3.8 Hz; H-12b, dd, J = 16.9, 9.0 Hz; H-13, dddd, J = 9.0, 6.5, 3.3, 3.2 Hz; OH-13, d, J = 3.2 Hz)] and suggested that the relative stereochemistry at these two centers might be unchanged. Observed NOEs between the pairs H-12a/H-10 and H-12b/H-13 were consistent with these findings. Furthermore, the NOEs of H-28a/H-12b and H-28b/H-13 were best accommodated by retention of tedanolide relative stereochemistry at C-14 during oxidation. We also observed transannular NOEs for H-2/H-12a and H-2/H-26, as Fusetani et al. have reported for 13-deoxytedanolide.[6] The NOE relationships are detailed in Table 1.

Fatty Acid Identification. In order to complete the identification of the active candidaspongiolides, the candidaspongiolide mixture (**3**) was subjected to a standard methanolysis technique, providing the corresponding fatty acid methyl esters (FAMEs). FAMEs were subjected to GC-MS analysis and identified by comparison to purchased standards. This analysis revealed $C_{14}-C_{18}$ carbon fatty acids with a high percentage of methyl-branched fatty acids (54%), confirming the information obtained from ^{13}C NMR analysis and in accord with other reports of sponge lipids.[8] Table 2 lists the acids present as C-28 esters in the candidaspongiolides and their relative abundance, based on GC peak intensities.

Enzymatic Hydrolysis. The presence of three esters—primary macrolide, primary fatty acid ester, and secondary acetate—in the candidaspongiolides (**3**), together with three ketones and an epoxide, rendered conventional hydrolysis an unlikely, if not impossible, avenue to the candidaspongiolide core. In contrast, the regio- and stereospecificity of enzymes represented a possible solution to this formidable challenge. Lipases and esterases have been used in organic chemistry for hydrolysis, transesterification, and asymmetric synthesis.[9,10] A lipase appeared to be preferable for the candidaspongiolide challenge, because these enzymes bind at the interface between aqueous and organic phases, exposing the active site only in the presence of lipid or organic solvent.[11] Enzymes have been used for many years for structure determination of primary metabolites such as saccharides and proteins, but only in the last few years has this selective tool been employed for secondary metabolites, typically for cleavage of glycoside bonds.[12,13] Studies on the kinetics of lipase-catalyzed reactions indicated a very positive increase of reaction rates with immobilized enzymes.[14,15] Therefore, we immobilized porcine lipase by adsorption on epoxy-activated Sepharose CL-4B.[16] After 66 h of exposure of the candidaspongiolides (**3**) to the immobilized enzyme, no more fatty ester was detected by TLC and the reaction was stopped simply by filtration through a sintered glass funnel. The ESIMS of the product presented a very prominent ion at *m*/*z* 529, which corresponded to a fragmentation α to a carbonyl between C-16 and C-17 of the candidaspongiolide core (**4**). Due to its insolubility in $CHCl_3$, all NMR experiments for **4** were obtained in acetone-d_6. (Assignments for **3** were also obtained in acetone-d_6. See Experimental Section and Supporting Information.) In the ^1H NMR spectrum, the lack of any signal at δ 1.2, characteristic of methylenes from long alkyl chains, indicated that cleavage of the fatty acid esters was complete. In addition, the upfield shift and coalescence of the H_2-28 AB pair to a broad singlet at δ 3.61 confirmed that the enzymatic reaction had affected the target ester at C-28. The comparison of ^{13}C NMR spectra (acetone-d_6) showed a downfield shift of C-28 from δ 64.6 (**3**, acetone-d_6) to δ 70.9 (**4**), corresponding to the transformation from an ester to a primary alcohol. The NMR spectra indicated no other modifications in the candidaspongiolide core (**4**), confirming a successful enzymatic hydrolysis (Table 3). Other small changes of chemical shifts observed in some signals corresponding to neighboring and nearby transannular positions (H-3, H-10, H-12, H-13, H-17, H-24, H-29a) could be due to a slight difference in conformation of the core macrolide (**4**).

Cytotoxicity. The candidaspongiolide mixture (**3**) and core (**4**) were potently cytotoxic in the NCI human disease-oriented, 60 tumor cell line assay, exhibiting a mean GI_{50} (total growth inhibition)[17] of 14 ng/mL for **3** and 42 ng/mL for **4** (Figure 1). In the 60-cell screen 13-deoxytedanolide (**2**) was more cytotoxic, with a mean GI_{50} of 0.09 ng/mL. On the average, melanoma cell lines were nearly 10-fold more sensitive than the other cell lines in the panel. Fusetani et al. have reported previously *in vitro* and *in vivo* activity for **2**,[6] and more recently this group has disclosed that **2** binds to the 60S subunit of the eukaryotic ribosome, apparently at the same site as pederin.[18] Fusetani's group also conducted a limited structure−activity relationship study of 10 derivatives of **2**, revealing the importance of the "southern hemisphere" of the molecule to the biological activity.[19] Interestingly, it is in this part of the molecule that the candidaspongiolides differ from **1** and **2**. Very recently, the Ireland group reported the structure of another tedanolide derivative, tedanolide C from a Papua New Guinea collection of *Ircinia* sp.[20] Tedanolide C was reportedly potently active against HCT-116, with an IC_{50} value of 57 ng/mL. In addition, the compound caused cell cycle arrest and accumulation of cells in the S-phase after a 24 h treatment at a concentration of 0.2 μg/mL.[20] Taken together, these data indicate that hollow fiber and *in vivo* xenograft studies of these new metabolites are warranted, once an adequate supply is established. Toward this end, it is encouraging that a total synthesis of tedanolide (**1**) has recently been published.[7]

It has been suggested[5] that the low yield of tedanolide (**1**) from *Tedania ignis* indicates that it might be derived from a microorganism. While the yields that were obtained in the present study are considerably higher than that obtained for tedanolide, the presence of compounds with the same macrolide nucleus in four sponges from four very different genera, from three widely separated sites, and the branched nature of the aliphatic esters in our isolates do tend to point to microorganisms as the source of these compounds. This possibility is under investigation at AIMS, and a recent paper has appeared in the literature identifying culturable symbionts from *C. flabellata*.[21]

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. IR spectra were obtained with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were recorded on either Bruker AC300P, Varian VXR 500, or Varian 500 MHz INOVA spectrometers using residual solvent as an internal reference. Low-resolution mass spectra were recorded on a Finnigan TXQ700 (CI, ammonia), while the high-resolution data were obtained from a Bruker APEX 47e FT-ICRMS in FAB mode using NOBA matrix. GC-MS analyses were performed on a Hewlett-Packard model 6890 gas chromatograph coupled to a Hewlett-Packard model 5973 mass spectrometer operated in scan mode with mass range of 40−500 amu, using a Supelco (Bellefonte PA) SP 2380, 100 m × 0.25 mm, 0.2 μm film thickness column. The injection conditions were splitless with 30 s vent time, helium carrier gas at 0.5 mL/min, oven program: 50 °C to 250 °C at 4 °C/min and held for 28 min to end of run. Injection port temp: 260 °C, transfer line temp: 280 °C. Samples were diluted

Table 3. NMR Spectroscopic Assignments (500 MHz, acetone-d_6) for Candidaspongiolide Core (**4**)

position.	δ_C mult.	δ_H mult. (*J* in Hz)	HMBC[a]	NOE
1	172.5 qC			
2	71.9 CH	3.75 d (9.0)	3	4, 32
3	84.4 CH	3.83 m	1, 2, 4, 5	4, 32
4	48.2 CH	3.19 dd (7.0, 9.5)	5, 6	2, 3, 6, 24
5	214.4 qC			
6	48.4 CH	3.42 m	5	4, 26
7	80.1 CH	5.40 d (10.5)	5, 6, 8, 9, 25, 26, 33	9, 25, 26
8	133.5 qC			
9	132.1 CH	5.46 dd (9.5, 1.0)	6, 26, 27	7, 10
10	45.5 CH	3.43 m	8, 11, 27	9
11	210.8 qC			
12	43.6 CH_2	a 2.73 dd (17.5, 9.0)	11, 13, 14	12b
		b 2.32 m	13, 14	12a, 13
13	68.9 CH_2	4.49 dd (9.0, 2.0)		12
14	85.2 qC			
15	215.9 qC			
16	47.8 CH	4.12 dt (11.5, 4.0)	15, 17, 18, 29	29a/b, 30
17	77.6 CH	3.25 d (10.5)	15, 18, 29, 30	19, 29b
18	63.2 qC			
19	66.0 CH	2.64 d (9.5)	17, 20, 21, 31	17, 20, 30, 31
20	31.6 CH	2.48 m	21, 22, 23, 31	19, 23, 30, 31
21	131.0 CH	5.36 dt (10, 1.5)	23, 31	
22	125.1 CH	5.49 m	21	
23	13.0 CH_3	1.65 dd (7.0, 1.5)	21, 22	20
24	22.9 CH_3	1.21 d (7.0)	3, 4, 5	4
25	14.5 CH_3	1.16 d (7.0)	5, 6, 7	7
26	10.3 CH_3	1.67 d (1.0)	7, 8, 9	6, 7
27	15.0 CH_3	0.97 d (6.5)	9, 10, 11	
28	70.9 CH_2	3.61 s	14	32
29	64.1 CH_2	a 4.34 dd (10.5, 4.0)	1, 15, 16, 17	16, 17, 29b
		b 3.90 dd (10.5, 11.5)		29a
30	11.1 CH_3	1.36 s	17, 18, 19, 20	16, 19, 20
31	18.4 CH_3	1.09 d (6.5)	19, 21	19, 20, 34
32	60.7 CH_3	3.40 s	3	2, 3, 28
33	170.0 qC			
34	20.6 CH_3	2.02 s	33	31

[a] HMBC correlations, optimized at 8 and 4 Hz, are from proton(s) stated to the indicated carbon.

in CH_2Cl_2, and 1 μL was injected. Preparative HPLC was performed on a Waters 600 pump controlled by MassLynx software. Postcolumn detection was accomplished by a parallel arrangement of a Micromass ZMD electrospray ionization (ESI) mass spectrometer (cone voltage = 30), a Waters 996 photodiode array (PDA), a Sedex 75 evaporative laser light scattering detector (ELSD), and a Kratos Spectroflow 980 spectrofluorimeter.

Collection and Taxonomy. Due to sparse distribution, and the small size of colonies, the sponge (Q66C0330) was collected opportunistically over a number of years by scuba in the central section of the Australian Great Barrier Reef (10−25 m depth) and kept frozen until extracted. [Note of caution: Contact dermatitis has afflicted some of those who have handled the sponge directly.] This sponge was subsequently identified as a new genus/species, *Candidaspongia flabellata*,[22] and the holotype (G 25081) has been deposited in the Queensland Museum. Two different collections from Papua New Guinea (0CDN1808 and 0CDN5955), initially identified as *Euryspongia* sp., were subsequently compared to specimens of *C. flabellata* and reclassified as *Candidaspongia* sp. The Papua New Guinea specimens are a darker color and have somewhat sharper conules, while the *C. flabellata* specimen from Australia had thicker fibers and a thicker sand coat on the surface. While the possibility that *C. flabellata* is variable enough to include fans with darker color, sharper conules on the surface, and differences in fiber thickness cannot be excluded, the Papua New Guinea specimens can be identified only to genus until more is known about this variability. Vouchers for the Papua New Guinea collections are maintained at the Smithsonian Sorting Center, Suitland, MD.

Extraction and Isolation. At AIMS, when sufficient material was available, the sponge was freeze-dried and extracted with CH_2Cl_2 until the increase in extract weight was less than 10%; in a typical example, 104 g (dry weight) of sponge yielded 1.55 g of extract. The CH_2Cl_2 extract was chromatographed on a silica gel flash column using hexane with increasing amounts of EtOAc. The 75% EtOAc fraction (210 mg) was further separated on normal-phase HPLC (RI detection) using hexane−EtOAc (3:2), yielding 125 mg of **3**.

Samples for the NCI were collected under contract in the same locations on the Great Barrier Reef, delivered frozen to the NCI, and subsequently extracted by a standard protocol. Briefly, the frozen sponge was shredded with dry ice in a meat grinder; the dry ice was allowed to sublime and the sponge tissue was soaked in distilled H_2O. The aqueous extract was removed by centrifugation and lyophilized. The sponge tissue was freeze-dried, extracted with CH_2Cl_2−MeOH (1:1), and then rinsed with MeOH. The combined organic extracts were evaporated under reduced pressure. Small amounts of the same mixture (**3**) were isolated from the NCI collection. At NCI, the Papua New Guinea (PNG) collections were also extracted using the standard NCI protocol. Materials from this collection were used to isolate the candidospongiolides in quantities large enough for enzymatic hydrolysis as follows.

A 500 mL aliquot of CH_2Cl_2 was added to the aqueous extract (50 g). The mixture was shaken, and the CH_2Cl_2 solubles were separated by filtration. The process was repeated three times, and the combined materials yielded 927.9 mg of gummy mass upon solvent evaporation. The CH_2Cl_2 solubles were divided into four fractions, and each one was permeated through a Sephadex LH-20 column with MeOH−MeCN (1:1). A total of 40 fractions (10 mL each) were collected, and four of those were combined (118.3 mg) after detecting the presence of candidaspongiolides by NMR spectroscopy. The final purification was performed employing gradient normal-phase HPLC (silica gel column, 1 × 25 cm; hexane−*i*PrOH−MeOH, t_0 = (94:3:3), t_{18} = (84:8:8), 5.2 mL/min; UV detection at 219 nm); 40 mg of candidaspongiolide mixture was obtained.

Candidospongiolide mixture (3): ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1; ^{1}H NMR (acetone-d_6) δ 5.50 (1H, dd, J = 9.5, 1.0 Hz, H-9), 5.47 (1H, dq, J = 11.0, 6.5 Hz, H-22), 5.36 (1H, d, J = 10.5 Hz, H-7), 5.33 (1H, dt, J = 11.0, 1.5 Hz, H-21), 4.34 (1H, m, H-29b), 4.33 (1H, d, J = 11.0 Hz, H-28a), 4.32 (2H, m, H-13), 4.13 (1H, d, J = 11.0 Hz, H-28b), 4.08 (1H, m, H-16), 4.07 (1H, m, H-29a), 3.86 (1H, dd, J = 7.0, 2.0 Hz, H-2), 3.72 (1H, dd, J = 9.5, 2.0 Hz, H-3), 3.53 (1H, dq, J = 9.5, 7.0 Hz, H-10), 3.37 (1H, dq, J = 10.5, 6.5 Hz, H-6), 3.35

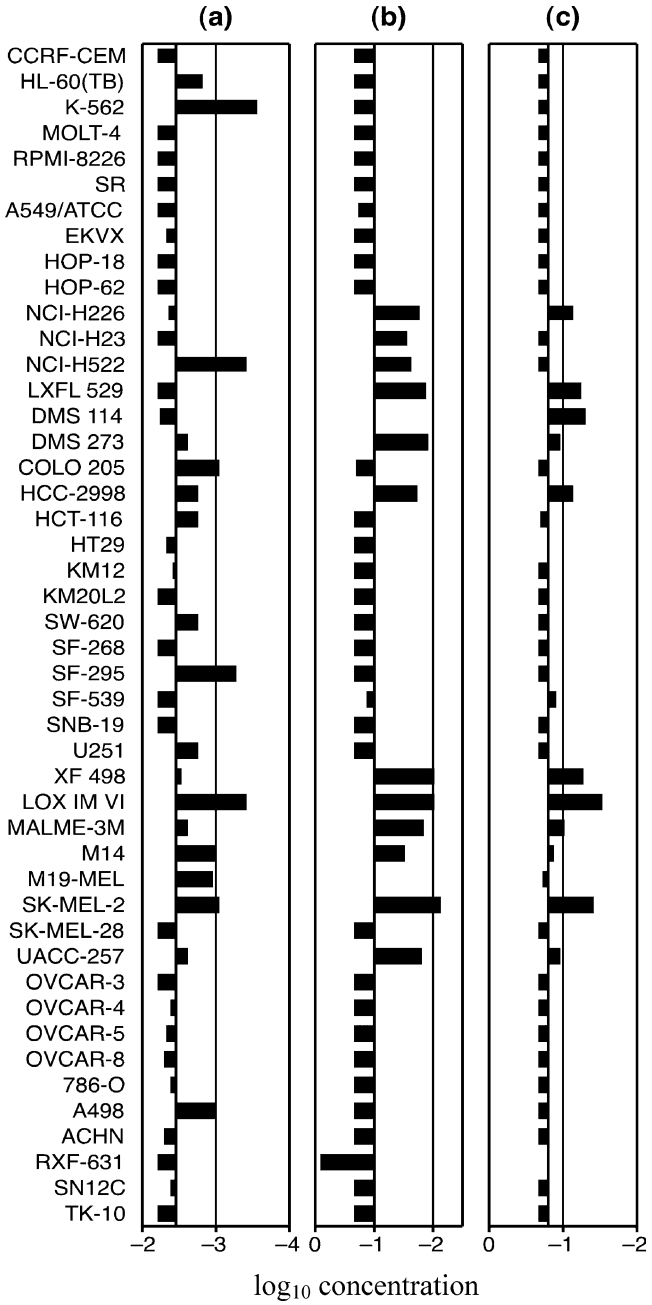


Figure 1. Mean bar graphs of the NCI's 60-cell-line screen at GI_{50} of (a) 13-deoxytedanolide (**2**), (b) candidospongiolide mixture (**3**), and (c) the candidospongiolide macrolide core (**4**). The line from which bars are displayed for each cell line is the mean log of concentration of the 50% growth inhibiting concentration (GI_{50}) for each compound.

(3H, s, H-32), 3.13 (1H, dq, J = 9.5, 7.5 Hz, H-4), 3.11 (1H, d br, J = 11.0 Hz, H-17), 2.74 (1H, dd, J = 16.5, 9.0 Hz, H-12a), 2.65 (1H, d, J = 9.0 Hz, H-19), 2.50 (1H, m, H-20), 2.49 (1H, br dd, J = 16.5, 2.0 Hz, H-12b), 2.26 (2H, t, J = 8.0 Hz, H-36), 2.01 (3H, s, H-34), 1.67 (3H, d, J = 1.5 Hz, H-26), 1.65 (3H, dd, J = 6.5, 2.0 Hz, H-23), 1.55 (2H, br, H-37), 1.41 (3H, s, H-30), 1.22 (3H, d, J = 7.5 Hz, H-24), 1.13 (3H, d, J = 6.5 Hz, H-25), 1.08 (3H, d, J = 6.5 Hz, H-31), 1.02 (3H, d, J = 7.0 Hz, H-27); ^{13}C NMR (acetone-d_6) δ 214.4 (s, C-5), 212.0 (s, C-15), 211.1 (s, C-11), 173.7 (s, C-35), 172.3 (s, C-1) 170.1 (s, C-33), 132.6 (d, C-9), 131.6 (s, C-8), 129.7 (d, C-21), 125.4 (dd, C-22), 84.2 (d, C-3), 82.6 (s, C-14), 81.2 (d, C-7), 77.8 (s, C-17), 72.5 (d, C-2), 69.4 (d, C-13), 66.5 (d, C-19), 64.6 (t, C-28), 63.2 (s, C-18), 63.1 (t, C-29), 61.7 (q, C-32), 49.1 (d, C-4), 48.7 (d, C-16), 48.3 (d, C-6), 46.1 (d, C-10), 43.4 (t, C-12), 34.1 (t, C-36), 31.6 (d, C-20), 25.3 (t, C-37), 20.7 (q, C-34), 18.4 (q, C-31), 16.2 (q, C-27), 15.0 (q, C-25), 14.3 (q, C-24), 13.2 (q, C-23), 11.0 (q, C-30) 10.6 (q, C-28).

Analysis of Fatty Acids. A 2.8 mg sample of the candidaspongiolide mixture (**3**) was dissolved in 0.1 mL of MeOH and mixed with 1.5 mL of 2 N methanolic KOH. Heptane (0.6 mL) was added to this mixture, which was then shaken vigorously for 10 min at room temperature (rt). From the heptane phase, 0.5 mg of fatty acid methyl esters (FAME) was recovered. The FAME composition was determined by GC-MS in comparison with retention times of standards using a bacterial acid mixture, supplemented by *anteiso* 16:0 and 17:0 methyl esters.

Enzymatic Hydrolysis. Employing a combination of methodologies,[16,23] a Sepharose matrix was prepared for the immobilization of porcine pancreatic lipase (PPL); 4 mL Sepharose CL-4B (Sigma) suspension was washed with 50 mL of H_2O and 20 mL of 0.5 M NaOH. The Sepharose was resuspended in 5 mL of 0.5 M NaOH and incubated with 1 mL of epichlorohydrin overnight. Epoxy-activated Sepharose was filtered and washed with H_2O and 0.1 M buffer phosphate at pH 8. A solution of 2 mg of PPL in 0.6 mL of 0.1 M buffer phosphate (pH 8) was added to the matrix and stirred for 4 h. To the enzymatic preparations was added a solution of the candidaspongiolide mixture (**3**, 3.5 mg in 0.9 mL of MeOH), and the mixture was mechanically shaken at rt for 66 h. The suspension was filtered through a sintered glass funnel, and the impregnated beads were washed with 50 mL of MeOH and 50 mL of H_2O. The liquid phase obtained was adjusted to pH 7 with phosphate buffer, and then the MeOH was removed under vacuum. The H_2O solution was loaded onto a C_{18} column, and it was eluted with a step gradient of MeCN−H_2O mixtures (3:7 to 1:0). Fractions collected between 30 and 50% MeCN showed the presence of macrolide core. After two consecutive preparative HPLC purifications (column: C18 Dynamax, 2.5 × 21 cm; method: gradient MeCN−H_2O, t_{0-5} = (4:6), t_{30} = (8:2), 10 mL/min), 0.7 mg of candidaspongiolide core (**4**) was obtained.

Candidaspongiolide core macrocycle (4): $[\alpha]^{25}_D$ +22 (*c* 0.45, MeOH); IR (NaCl) ν_{max} 3447, 2951, 2923, 1734, 1717, 1653, 1457, 1386, 1336 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRMS *m*/*z* 707.32525 [MNa$^+$], calcd for $C_{34}H_{52}NaO_{14}$, 707.32547.

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Supporting Information Available: 1D and 2D NMR spectra of the candidaspongiolide mixture (**3**) and the core macrocycle (**4**) and table of ^{1}H NMR assignments in $CDCl_3$ for candidspongiolide mixture (**3**) and tedanolide (**1**, isolated[5] and synthetic[7]). This material is available free of charge via the Internet at http://pubs.acs.org.

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