Comparison of Fascaplysin and Related Alkaloids: A Study of Structures, Cytotoxicities, and Sources

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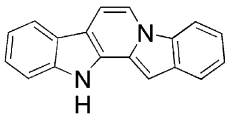
Received February 4, 2004

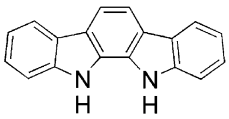
The fascaplysin class of compounds have been further investigated from six organisms consisting of four sponge collections (*Fascaplysinopsis reticulata*) and two tunicate collections (*Didemnum* sp.). This work is an extension of an earlier communication and reports the isolation of 12 new fascaplysin derivatives: 10-bromofascaplysin (**7**), 3,10-dibromofascaplysin (**8**), homofascaplysate A (**9**), homofascaplysin B-1 (**11**), 3-bromohomofascaplysins B (**12**), B-1 (**13**), and C (**15**), 7,14-dibromoreticulatine (**17**), reticulatol (**20**), 14-bromoreticulatol (**21**), and 3-bromosecofascaplysins A (**22**) and B (**23**), along with known compounds: fascaplysin (**1**), reticulatine (**4**), 3-bromofascaplysin (**6**), and homofascaplysin C (**14**). Selected compounds were screened in a cell-based cytotoxicity assay with compounds **1**, **6**, and fascaplysin A (**24**) also screened in the NCI 60 cell line panel. A biogenetic pathway for the brominated fascaplysins and brominated related alkaloids is proposed and discussed.

Two groups of closely related bis-indole natural products are a topic of recent research because their members have diverse structures, though based on a similar scaffold, and exhibit distinct biological properties. Two isomeric core ring systems of formula $C_{18}H_{12}N_2$ are distinguished by the relative position of the indole nitrogens. The first structural family has a 12*H*-pyrido[1,2-*a*;3,4-*b*′]diindole pentacyclic framework shown in structure **A** and is headed by the fascaplysins[1] and the homofascaplysins,[2] which are exclusively of marine origin. The other isomeric type has an indolo[2,3-*a*]carbazole pentacyclic system shown in structure **B** and includes the tjipanazoles[3] from a terrestrial cyanobacteria and the staurosporines isolated from terrestrial bacteria[4] and marine organisms.[5] From a mechanistic view, Van Vranken has outlined how a ditryptophan might cyclize via two distinct pathways into **A** and **B**, suggesting an intriguing similarity for the early steps of their formation.[6]



12*H*-pyrido[1,2-*a*;3,4-*b*′]diindole

A



indolo[2,3-*a*]carbazole

B

The members of both series have been the subjects of total syntheses and limited biosynthetic pathway analysis, and their biological properties have been scrutinized. From the perspective of bioactivity, it is the derivatives of **B** that have been more intensely studied in recent years. For example, lead compounds are currently in clinical development against DNA topoisomerase I (rebeccamycin analogue)[7] and protein kinases (staurosporine analogues).[8] We believe the compounds of **A** have similar potential, and recently, in a brief communication, we summarized[9] the structures and cytotoxicities of three new fascaplysins. The purpose of this present account is to now extend these observations through a comprehensive report.

The current literature contains inconsistent nomenclature for pentacyclic and tetracyclic natural products considered related to fascaplysin (**1**). As a unifying concept, we prefer to group these natural products into the three classes shown in Figure 1. The two structural variants most closely related to **1**, defined here as a type **Ia** framework, are (a) type **Ib**, shown in the structure of homofascaplysin A (**2**),[2,10] and (b) type **Ic**, illustrated by homofascaplysin B (**3**).[10] The distinction among **Ia**−**Ic** is based on the changes in the extent of conjugation present in the fused pentacyclic chromophore. A single bond disconnection severing ring D of the **A** scaffold is the basis of defining the other related classes comprised of a type **II** skeleton, as exemplified by reticulatine (**4**),[11] and a type **III** system such as secofascaplysin A (**5**).[10] The β-carboline ring system forms the signature core for both of these latter classes, which are distinguished by differences in the oxidation state of the C ring.

The succession of structural modifications represented in **1**−**5** along with the potential to exploit their promising biological properties prompted a reinvestigation of fascaplysin-containing sponges (by the UCSC group) and tunicates (by the OU group). The goal was to probe the antiproliferative properties as a function of structure and to examine the most potent analogues in advanced assays. We now report on the continuation of our work described in a communication[9] where we disclosed the isolation and characterization of three new compounds, 3-bromofascaplysin (**6**), 14-bromoreticulatine (**16**), and 14-bromoreticulatate (**19**) as well as reticulatate (**18**), previously described as a semisynthetic product of **1**.[12] Outlined below is the isolation and characterization of 12 novel fascaplysin derivatives, **7**−**9**, **11**−**13**, **15**, and **20**−**23**, in addition to the known compounds **1**, **4**, **6**, and **14**. Also discussed are the cytotoxicities of select compounds in a disk diffusion soft agar assay and the NCI 60 cell line panel.

Results and Discussion

To understand the strategy taken in the isolation work, it is relevant to provide a perspective of the biogeographical distribution of the pentacyclic and tetracyclic alkaloids related to **1**. The compilation of Table 1 shows **1**−**5**, plus

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10.1021/np049935+ CCC: $27.50
Published on Web 04/10/2004

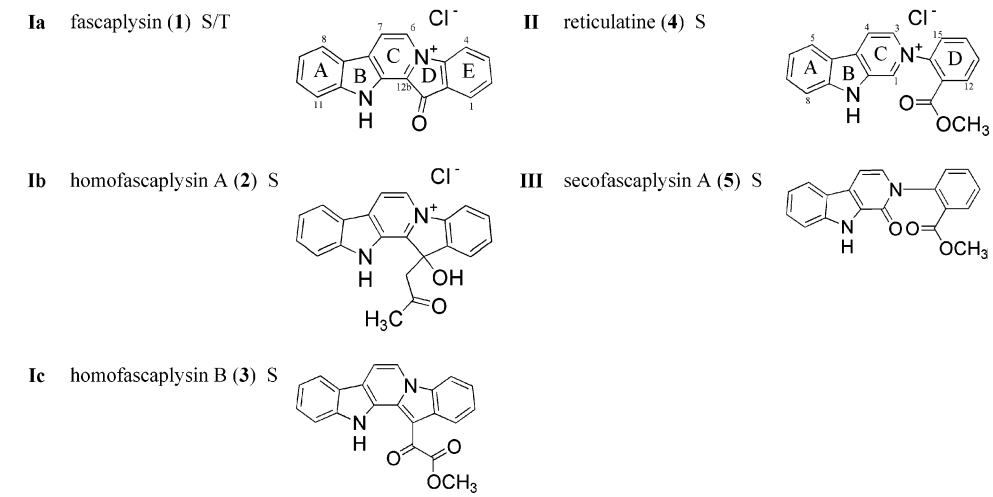


Figure 1. Three known classes of fascaplysins and related alkaloids of sponge (S) and tunicate (T) origin.

Table 1. Summary of Fascaplysin and Related Alkaloids and Aplysinopsins from Thorectidae Sponges and Tunicate Sources[a]

coll. no.	taxonomic identification	collection site[b]	alkaloids present	ref
	Fascaplysinopsis reticulata	GBR	aplysinopsins	19
	Fascaplysinopsis sp. (Ayling)	Fiji	**1**	1
87057	*F. reticulata* (Diaz)	Fiji	**1**, **2**, **3**, **5**, **14**	10
88106	*F. reticulata* (Diaz)	Fiji	**1**, **3**, **5**, **14**	10
89051	*F. reticulata* (Diaz & van Soest)	Fiji	**1**, **3**, **4**, **5**, **14**	10, 11
	Fascaplysinopsis sp. (Harper)	Palau	**1**	14
	Hyrtios cf. *erecta* (Hooper)	Fiji	**1**, **2**	15
	Hyrtios erecta	Okinawa	aplysinopsins	20
0CDN5079	*Thorectandra* sp. (Kelly)	Palau	**1**, **10**	2, 16
89128	*F. reticulata* (Diaz)	Fiji	**1**, **4**, **18**	9
95604	*F. reticulata* (Diaz)	Indonesia	**6**, **16**, **19**	9
0CDN3230	*F. reticulata* (Kelly)	Phillippines	aplysinopsins	13
0CDN5714	*Thorectandra* sp. (Kelly)	PNG	aplysinopsins	13
	Didemnum sp. (Monniot)	Fiji	**1**	17
89106	*Tunicate*[c]	Solomon Islands	**1**	18
115T93	*Didemnum* sp. (Monniot)	Micronesia	**1**, **6**	9
10IN95	*Didemnum* sp. (Monniot)	Indonesia	**1**, **6**	9

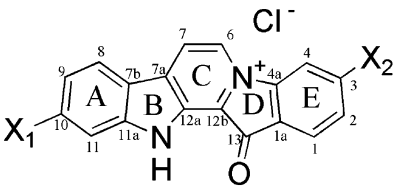
[a] Structures of compounds shown in Figures 1 and 2. [b] GBR, Great Barrier Reef; PNG, Papua New Guinea. [c] No taxonomic data.

an additional six known analogues, and the unrelated aplysinopsins, derived from monomeric tryptophan, alongside their sponge and tunicate sources. All three classes of fascaplysins (**I**−**III**) have been reported from different Thorectidae sponges collected from across the Indo-Pacific region. These include *Fascaplysinopsis reticulata* (Bergquist 1980) from Fiji and Indonesia (**I**−**III**);[9−11,13] *Fascaplysinopsis*. sp. (Bergquist 1980) from Fiji and Palau (**Ia**);[1,14] *Hyrtios* cf. *erecta* (Duchassaing & Michelotti 1864) from Fiji (**Ia** and **Ib**);[15] and *Thorectandra* sp. (Lendenfeld 1889) from Palau (**Ia** and **Ib**).[2,16] The type **Ia** fascaplysins have also been reported from the tunicate *Didemnum* sp. (Monniot & Monniot 1997) collected from a variety of Indo-Pacific locations including Fiji,[17] Solomon Islands,[18] Micronesia,[9] and Indonesia.[9] A much broader phyletic occurrence is observed for the aplysinopsins. These compounds have been isolated by two separate laboratories from *Fascaplysinopsis reticulata*[13,19] and from the sponges *Thorectandra* sp.,[13] *Hyrtios erecta*,[20] *Verongia spengelii* (syn. *Aplysina spengelii*),[21] *Smenospongia aurea*,[22] and *Dercitus* sp.,[23] as well as scleractinian corals of the genera *Tubastrea*,[24] *Astroides*,[25] and *Dendrophyllia*.[26]

The organisms included in this study were selected both from samples in our repositories (UCSC and OU) and from collections provided by the NCI-DTP. The isolation work reported below began after LCMS screening of Kupchan-type solvent partition fractions[27] from 12 separate sponge specimens of *Fascaplysinosis reticulata* (UCSC), two *Didemnum* sp. tunicates (OU), and two oils provided by the NCI-DTP. During the reisolation it was important to be able to rapidly dereplicate[28] among the 11 known fascaplysin analogues,[1,9−11,15] which, as a function of class, provide diagnostic NMR signals shown in Table 2. Searching the ^{1}H and ^{13}C NMR spectra of semipure fractions for these characteristic signals was followed by inspection of the MS for the molecular ion or key fragment ions. Four sponge fractions and two tunicate fractions were prioritized for fractionation based on the presence of alkaloids possessing molecular weights that did not correspond to any known fascaplysin. The isolation outlined in Chart S1 (see Supporting Information) provided both known and new fascaplysins. Some of the fractions were purified to yield the known compounds fascaplysin (**1**),[1] reticulatine (**4**),[11] 3-bromofascaplysin (**6**),[9] and homofascaplysin C (**14**).[10]

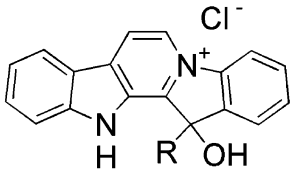
A variety of chromatographic methods ranging from silica gel, Sephadex, and preparative reversed-phase HPLC were used to isolate an additional 16 compounds. Selected fractions were then examined by mass spectrometry to identify those containing materials of further interest. The final purification involved semipreparative HPLC and yielded 3-bromofascaplysin (**6**), 14-bromoreticulatine (**16**), reticulatate (**18**), and 14-bromoreticulatate (**19**), previously described in the communication.[9] These were accompanied by 12 new compounds shown in Figure 2, including 10-

Type **Ia**

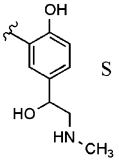


3-bromofascaplysin (**6**) X_1 = H, X_2 = Br S/T
10-bromofascaplysin (**7**) X_1 = Br, X_2 = H S
3,10-dibromofascaplysin (**8**) X_1 = X_2 = Br S

Type **Ib**

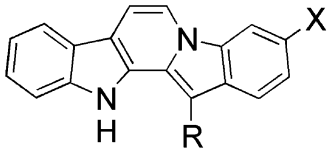


homofascaplysate A (**9**) R = $CH2CO_2H$ S/T



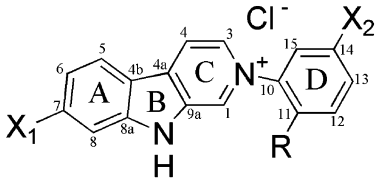
thorectandramine (**10**) R = S

Type **Ic**



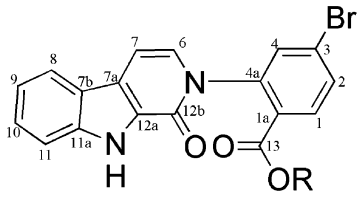
homofascaplysin B-1 (**11**) R = $C(O)C(O)OCH_2CH_3$, X = H T
3-bromohomofascaplysin B (**12**) R = $C(O)C(O)OCH_3$, X = Br T
3-bromohomofascaplysin B-1 (**13**) R = $C(O)C(O)OCH_2CH_3$, X = Br T
homofascaplysin C (**14**) R = C(O)H, X = H S/T
3-bromohomofascaplysin C (**15**) R = C(O)H, X = Br T

Type **II**



14-bromoreticulatine (**16**) R = $C(O)OCH_3$, X_1 = H, X_2 = Br S
7, 14-dibromoreticulatine (**17**) R = $C(O)OCH_3$, X_1 = X_2 = Br S
reticulatate (**18**) R = C(O)OH, X_1 = X_2 = H S
14-bromoreticulatate (**19**) R = C(O)OH, X_1 = H, X_2 = Br S
reticulatol (**20**) R = OH, X_1 = X_2 = H S
14-bromoreticulatol (**21**) R = OH, X_1 = H, X_2 = Br S

Type **III**



3-bromosecofascaplysin A (**22**) R = CH_3 S
3-bromosecofascaplysin B (**23**) R = H S

Figure 2. Summary of additional fascaplysins and related alkaloids of sponge (S) and tunicate (T) origin.

Table 2. Diagnostic NMR Shifts of the Different Fascaplysin Types

	NMR range				
type	δ_H 8.5−9.5	δ_H 7.0−8.5	δ_C 150−200	δ_C 60−150	example
Ia[a]	~9.4 d (H-6) ~9.0 d (H-7)		~184 s (C-13)		**1**
Ib[a]	~9.3 d (H-6) ~8.8 d (H-7)			~80 s (C-13)	**2**
Ic[b]		~8.4 d (H-6) ~7.8 d (H-7)		~104 s (C-12b)	**3**
II[a]	~9.3 d (H-1) ~8.8 d (H-4) ~8.6 d (H-3)		~164 s (C-16)		**4**
III[a]		~7.2 d (H-6) ~7.1 d (H-7)	~156 s (C-12b)		**5**

[a] ^{1}H NMR signals observed in MeOH-d_4. [b] ^{1}H NMR signals observed in $CDCl_3$.

bromofascaplysin (**7**), 3,10-dibromofascaplysin (**8**), homofascaplysate A (**9**), homofascaplysin B-1 (**11**), 3-bromohomofascaplysins B (**12**), B-1 (**13**), and C (**15**), 7,14-dibromoreticulatine (**17**), reticulatol (**20**), 14-bromoreticulatol (**21**), and 3-bromosecofascaplysins A (**22**) and B (**23**).

Establishing molecular formulas for the 12 new fascaplysins was the first step in their structure elucidation. The next action was to search for the diagnostic resonances of Table 2 for the C-ring protons H-6 and H-7 indicative of type **Ia** versus **Ib**, **Ic**, or **III** frameworks and the C-ring protons H-1, H-3, and H-4, diagnostic for the **II** system. Attention then turned to using high-field and low-field ^{13}C and ^{1}H NMR data as a starting point to rapidly categorize structural variations within the new analogues. Overall,

Table 3. NMR Data[a] for 10-Bromofascaplysin (**7**), 3,10-Dibromofascaplysin (**8**), Homofascaplysate A (**9**), Homofascaplysin B-1 (**11**), 3-Bromohomofascaplysin B (**12**), 3-Bromohomofascaplysin B-1 (**13**), and 3-Bromohomofascaplysin C (**15**)

	7[b]			**8**				**9**[b]		**11**[d]	**12**[d]	**13**[d]	**15**[d]
position	δ_C	δ_H (J in Hz)	HMBC	δ_C [c]	δ_H (J in Hz)[b]	δ_H (J in Hz)[c]	HMBC[c]	δ_C	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1a	124.1			130.6				139.8					
1	125.5	8.06 dd (0.9,7.7)	3, 4, 13	127.0	7.96 d (1.2)	8.02 d (8.2)	1a, 4a, 13	126.0	7.93 ddd (0.6, 1.4,7.5)	8.15 d (7.8)	7.60 m	7.62 d (9.0)	8.05 d (7.5)
2	131.4	7.76 ddd (7.5,7.5, 0.6)	1a, 3, 4	134.4	7.96 d (1.2)	7.98 dd (1.1, 8.0)	3	131.6	7.69 ddd (1.0, 7.5, 7.5)	7.37 t (7.8)	7.60 m	7.62 d (9.0)	7.63 dd (1.5, 7.5)
3	136.9	7.98 ddd (7.8,7.8, 1.0)	1, 4a	123.3				131.7	7.74 ddd (1.3, 7.8, 7.8)	7.57 t (7.8)			
4	115.0	8.33 d (7.9)	1a, 2	119.5	8.70 t (0.9)	8.91 d (1.4)	1a, 2, 3, 4a	115.1	8.23 d (8.0)	7.75 t (7.8)	8.18 s	8.18 d (2.0)	8.17 d (1.5)
4a	147.3			148.0				142.3					
6	126.6[e]	9.38 d (6.4)	7, 7a, 12b	127.6	9.39 d (6.4)	9.63 d (6.4)	7, 7a, 12b	124.2	9.28 d (6.6)	8.45 d (6.8)	8.36 d (7.0)	8.36 d (7.0)	8.28 d (7.0)
7	119.9	8.94 d (6.1)	6, 12a	120.7	8.97 d (6.4)	9.22 d (5.9)	6, 12a	118.1	8.81 d (6.5)	7.84 d (6.8)	7.86 d (7.0)	7.85 d (7.0)	7.74 d (7.0)
7a	140.8			140.3				136.4					
7b	118.8			118.7				121.5					
8	125.0	8.39 d (8.5)	7a, 10, 11a	126.2	8.41 d (8.4)	8.53 d (8.7)	10, 11a	124.5	8.47 ddd (1.0, 1.0,8.0)	8.02 d (7.8)	8.15 d (8.0)	8.15 d (8.0)	8.11 d (8.0)
9	126.6[e]	7.67 dd (1.5,8.5)	7b, 11	126.5	7.70 dd (1.6, 8.5)	7.75 dd (1.4, 8.7)	7b	123.4	7.51 ddd (1.5, 6.5,8.1)	7.46 t (7.8)	7.38 t (8.0)	7.37 t (8.0)	7.35 t (8.0)
10	128.8			128.1				133.6	7.84 m	7.52 t (7.8)	7.59 t (8.0)	7.58 t (8.0)	7.56 t (8.0)
11	116.2	8.01 d (1.4)	7b, 9, 10	147.7	8.03 d (1.2)	7.98 d (1.4)	7b, 8, 10	114.1	7.84 m	7.74 (7.8)	7.76 d (8.0)	7.75 d (8.0)	7.75 d (8.0)
11a	147.8			116.5				146.6					
12a	132.0			131.3				132.4					
12b	122.7			123.6				146.0					
13	181.6			181.2				80.7					
14								45.3	4.07 d (17.0), 3.74 d (17.0)				10.25 s
15								176.0					
OCH_2										4.56 q (6.8)		4.56 q (7.0)	
CH_3										1.46 t (6.8)		1.46 t (7.0)	
OCH_3											4.08 s		
NH										12.5 bs	12.5 bs	12.5 bs	12.5 bs

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b] Recorded in MeOH-d_4. [c] Recorded in DMSO-d_6. [d] Recorded in CD_2Cl_2. [e] Assignments can be switched.

Table 4. NMR Data[a] for 7,14-Dibromoreticulatine (**17**), Reticulatol (**20**), 14-Bromoreticulatol (**21**), 3-Bromosecofascaplysin A (**22**), and 3-Bromosecofascaplysin B (**23**) in MeOH-d_4

	17			**20**			**21**				**22**			**23**		
position	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC	position	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC
1	130.9	9.45 dd (0.6, 1.4)	4a	130.6	9.28 dd (1.4, 0.6)	3, 4, 10	130.5	9.31 dd (0.8, 1.4)	3	1a	127.8			128.3		
3	134.2	8.66 dd (1.4, 6.6)	1, 4, 4a, 10	133.9	8.59 dd (1.3, 6.5)	1, 4, 4a	133.8[b]	8.60 dd (1.4, 6.5)	1, 4, 4a	1	132.2[c]	7.97 d (8.3)	3, 4a, 13	132.5	8.04 d (8.6)	3, 4a, 13
4	116.7	8.79 dd (0.6, 6.5)	4b, 9a	116.8	8.78 dd (0.6, 6.3)	1, 4a, 4b, 9a	116.8	8.78 dd (0.7, 6.5)	4b, 9a	2	131.9[c]	7.78 dd (2.0, 8.3)	1a, 4, 4a, 13	131.9[e]	7.80 dd (2.0, 8.4)	1a, 3, 4, 4a
4a	133.8			133.6			133.8[b]			3	126.6			126.2		
4b	118.5			119.5			119.5			4	132.1[c]	7.70 d (2.0)	1a, 2, 4a, 13	132.1[e]	7.69 d (2.0)	1a, 2, 3, 4a, 13
5	124.7	8.43 dd (0.6, 8.5)	7, 8, 8a	123.1	8.49 ddd (1.0, 1.0, 8.0)	4b, 7, 8, 8a	123.2	8.49 ddd (1.0, 1.0, 8.2)	4b, 7, 8, 8a	4a	141.5			141.8		
6	125.7	7.67 dd (1.6, 8.5)	7b, 8	122.0	7.53 m	4b, 7, 8	122.1	7.52 ddd (1.0, 7.0, 8.1)	4b, 5, 7, 8,	6	128.0[d]	7.21 d (6.9)	4a, 7, 7a, 12b	128.1	7.22 d 6.9	4a, 7, 7b, 12b
7	127.1			132.6	7.87 ddd (1.2, 7.0, 8.4)	5, 8a	132.8	7.87 ddd (1.2, 7.0, 8.2)	5, 8a	7	101.5	7.18 d (6.9)	12a	101.4	7.19 d (6.9)	7b, 12a, 12b
8	115.8	8.05 dd (0.5, 1.7)	4b	112.6	7.81 ddd (0.9, 0.9, 8.5)	4b, 6	112.7	7.82 ddd (0.9, 0.9, 8.4)	4b, 6	7a	125.5			125.5		
8a	145.7			145.1			145.2			7b	122.1			122.0		
9a	135.0			135.1			134.9			8	120.8	8.01 ddd (1.0, 1.0, 8.0)	7b, 10, 11a	120.8	8.01 ddd (1.0, 1.0, 8.0)	7a, 7b, 10, 11a
10	143.5			131.3			132.0			9	119.6	7.23 ddd (1.0, 7.1, 8.0)	8, 11	119.9	7.23 ddd (1.0, 7.1, 8.0)	7b, 11
11	124.8			151.0			150.0			10	126.9	7.45 ddd (1.2, 7.0, 8.3)	8, 11a	126.8	7.45 ddd (1.2, 7.2, 8.4)	8, 11a
12	133.2	8.24 d (8.6)	10, 14	117.0	7.18 dd (1.3, 8.2)	10, 11, 14	118.5	7.12 d (8.9)	10, 11, 14	11	112.1	7.56 ddd (0.8, 0.8, 8.3)	9	112.0	7.56 ddd (0.8, 0.8, 8.3)	7b, 9
13	134.7	8.09 dd (2.0, 8.5)	11, 15	132.0	7.53 m	11, 12, 15	134.9	7.68 dd (2.4, 8.9)	11, 15, 14	11a	140.0			139.9		
14	127.6			119.9	7.14 ddd (1.3, 7.7, 7.7)	10, 11, 12, 15	110.7			12a	128.0[d]			127.2		
15	131.2	8.14 d (1.8)	11, 13, 14	126.4	7.61 dd (1.6, 7.8)	11, 13	129.4	7.88 d (2.5)	10, 11, 13, 14	12b	155.8			156.0		
16	163.2									13	164.8			165.8		
OCH_3	51.9	3.68 s								OCH_3	51.4	3.58 s	13			

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b–e] Assignments can be switched.

the 10 different diagnostic NMR shifts shown in Table 2 allowed rapid categorization of 12 compounds among the five structural categories (**I**−**III**).

Two of the halogenated compounds were identified as belonging to the type **Ia** group. These were 10-bromofascaplysin (**7**) and 3,10-dibromofascaplysin (**8**), and their structures were deduced by comparing their molecular formulas and NMR data with those of closely related compounds **1** and **6**. The molecular formula of **6** and **7** ($C_{18}H_{10}BrN_2O$) showed they were isomeric, and the ^{1}H NMR analysis of the spin systems of their A and E rings was used to establish the location of the Br. Similarly, the molecular formula of **8**, $C_{18}H_9Br_2N_2O$, identified it as a dibromo analogue of nonbrominated **1** or monobrominated **6** and **7**. Comparing the NMR data in MeOH-d_4 and DMSO-d_6 of these four compounds allowed the Br atoms to be confidently placed at C-3 and C-10 for compound **8**.

The single example of a new type **Ib** compound isolated proved to be homofascaplysate A (**9**). The alkaloid portion of this salt, assumed to have a Cl^- counterion, possessed a molecular formula differing from that of **2** by loss of CH_2 and addition of O. The ^{13}C and ^{1}H NMR resonances for the pentacyclic system of **9** were all observable. The different functional group attached at C-13 was identified as a $-CH_2$-CO_2H from the methylene AB spin system at δ 4.07/3.74 (H_2-14) and the carbonyl group at δ 176 (C-15). The connectivity between C-13, C-14, and C-15 was further confirmed by the HMBC correlations from H_2-14. Unlike all of the other compounds isolated, **9** possessed a single chiral center and its $[\alpha]_D = +14°$ indicated that material enriched in a single enantiomer had been isolated.

Extending the structural theme of homofascaplysin B (**3**) were four **Ic** analogues, **11**, **12**, **13**, and **15**, which were exclusively isolated from a tunicate. The first of these compounds, homofascaplysin B-1 (**11**), was found to have a molecular formula of $C_{21}H_{14}N_2O_3$. The ^{1}H NMR data for **11** closely matched that of **3**; however, the OCH_3 singlet present in **3** was replaced by an OC_2H_5 group identified from ^{1}H NMR resonances that included a two-proton quartet at δ_H 4.56 and MS fragmentation ion peaks *m*/*z* 283 and 255 corresponding to the loss of C(O)OEt and C(O)C(O)OEt, respectively. The brominated analogues of **3** and **11** were also isolated and include 3-bromohomofascaplysin B (**12**) with a formula $C_{21}H_{13}BrN_2O_3$ and 3-bromohomofascaplysin B-1 (**13**) with a formula $C_{22}H_{15}BrN_2O_3$. The ^{1}H NMR data for **12** and **13** revealed three aromatic spin systems consisting of one ABCD (H-8−H-11), one AB (H-6−H-7), and one ABX (H-1−H-4), respectively. The attachment of the bromine in **12** and **13** was assigned to C-3 on the basis of NOESY correlations between H-4 and H-6. The last compound characterized was 3-bromohomofascaplysin C (**15**), with a molecular formula of $C_{19}H_{11}BrN_2O$. The ^{1}H NMR data clearly showed that aromatic ring H-3 of homofascaplysin C (**14**) was replaced in **15** by Br, and NOESY data verified the assignment of Br to C-3.

Attention shifted next to the characterization of three type **II** compounds, and the known metabolites reticulatine (**4**), 14-bromoreticualatine (**16**), reticulatate (**18**), and 14-bromoreticulatate (**19**) provided important reference data. Characterization of 7,14-dibromoreticulatine (**17**) began by defining the molecular formula, $C_{19}H_{13}Br_2N_2O$, from the HRESITOFMS *m*/*z* 458.9353 $[M]^+$ peak. Side-by-side comparison of its NMR resonances (assigned from ^{1}H and ^{13}C NMR and HMBC data) to those of **4** and **16** confirmed the placement of the two bromines at C-7 and C-14, respectively. Two closely related compounds, reticulatol (**20**), with a formula $C_{17}H_{13}N_2O$ based on a HRESITOFMS *m*/*z* 261.1041 $[M]^+$, and 14-bromoreticulatol (**21**), with a formula $C_{17}H_{12}BrN_2O$ based on a HRESITOFMS cluster centered at *m*/*z* 339.0085 $[M]^+$, were resolved next. These formulas differed from that of **4** and **16**, respectively, by the loss of C_2H_2O and suggested that a phenol had replaced the methyl ester functionality. Additional data to support the D-ring assignment involved comparison of ^{1}H and ^{13}C NMR data for **20** versus **4** and **21** versus **16** (see Table S2). This revealed a large upfield shift difference at H-12, H-14 (in **20**), C-10, C-12, and C-14 as well as the large downfield shift difference at C-11.

The last compounds characterized proved to be new **III** types related to secofascaplysin (**5**).[10] These consisted of 3-bromosecofascaplysins A (**22**) and B (**23**), both isolated as yellow solids. As expected from the NMR data, molecular formulas $C_{19}H_{13}BrN_2O_3$ and $C_{18}H_{11}BrN_2O_3$ were established for **22** and **23** via HRESITOFMS (*m*/*z* 397.0160 $[M + H]^+$ and 383.0102 $[M + H]^+$, respectively); they were closely related. It was quickly recognized that **22** was the C-3 bromo analogue of **5**. The ^{1}H and ^{13}C NMR data for rings A−C of **22** closely matched those of **5**, leaving the placement of Br to the D ring. Analysis of HMBC correlations, including H-1 to C-13, H-1 to C-4a, H-1 to C-3, H-2 to C-1a, and H-4 to C-1a, confirmed the placement of the Br at C-3. Subsequent comparison of the spectral properties in Table 4 of **23** with **22** supported the acid/ester relationship assigned for this pair.

We along with other groups have speculated that the fascaplysins arise through the modification of a ditryptophan.[10] However, recently the biosynthetic machinery responsible for the production of rebeccamycin, a bishalogenated indole[2,3-*a*]carbazole, has been mapped and the gene responsible for the condensation of two tryptophans has been identified.[29] The structural knowledge provided by the 19 compounds isolated in this study (see Chart S1) and especially the brominated analogues provided the basis to reexamine their overall biogenetic relationship. As shown in Scheme 1 if one of the two tryptophans is brominated, that would lead to **6**, **7**, **12**, **13**, **15**, **16**, **19**, and **21**−**23**, or if both are brominated, **8** and **17** would arise. A ring opening of **6** would yield type **II** and **III** compounds including **19**, which is the likely precursor for **21** with the loss of CO and **23** with oxidation. Methylation of **19** and **23** would produce **16** and **22**, respectively. Interestingly, no derivatives of **7** were observed in this study. We believe a brominated homofascaplysate A (**9**) provides a route from **6** to the brominated type **Ic** compounds, and LCMS data identified a very minor component with a mass corresponding to this intermediate. A brominated α-keto acid is the likely precursor to **12**, **13**, and **15**, with loss of CO_2 yielding **15**.

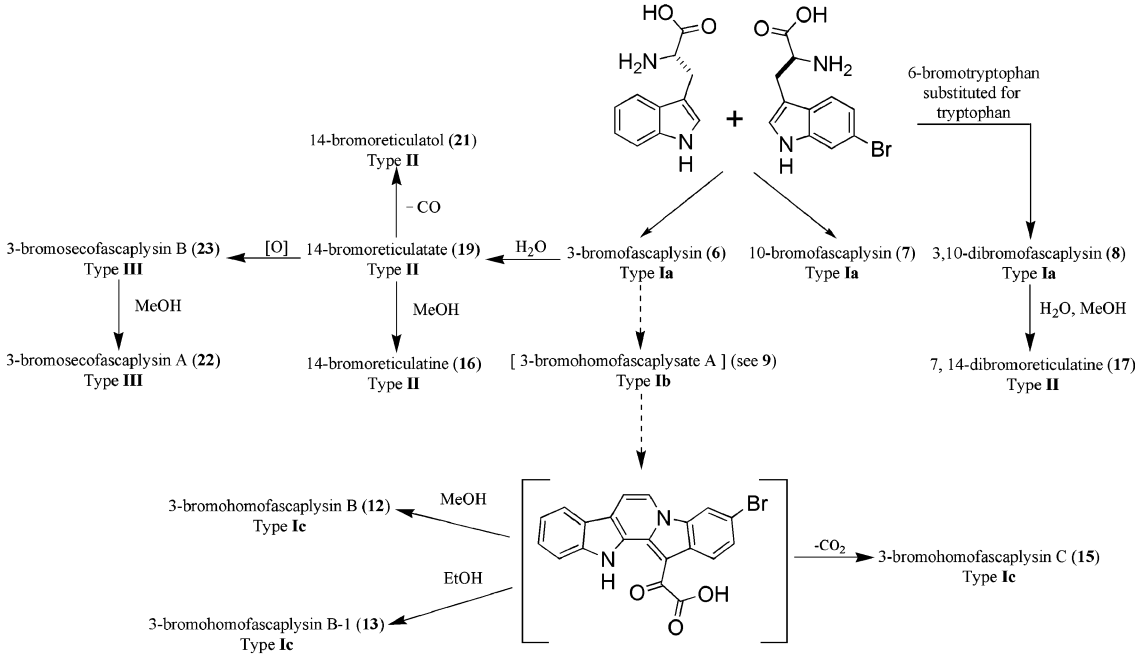
The major biological properties discovered for fascaplysin (**1**) involve selective inhibition of cyclin dependent kinase 4[30] and DNA intercalation.[31] The cytotoxic properties of **1**, **4**, **6**, **7**, and **16**−**21** were assessed against murine and human tumor cell lines using the disk diffusion soft agar assay[32] as shown in Table 5 (also see Table S2), with significant selectivity shown in bold. To calibrate our findings, bengamide E[33] was included as a standard. Fascaplysin (**1**), the most potent compound tested, exhibited murine tumor selectivity. However, 10-bromofascaplysin (**7**) showed the most interesting activity and had a potency similar to that of **1** but with human solid tumor selectivity, which **1** lacks. Reticulatine (**4**) displayed moderate activity and retained murine tumor selectivity. The only compound selective for both murine and human tumors was reticulatate (**18**). Another significant result of Table 5 is that reticulatol (**20**) showed significant selectivity for

Table 5. Zone Unit Differentials in the Disk Diffusion Soft Agar Colony Formation Assay[a]

		murine tumor selectivity[b]		human tumor selectivity[b]
compound	conc, μg/disk	Z_{C38}-Z_{L1210}	Z_{C38}-$Z_{CFU(GM)}$	$Z_{H116\backslash H125}$-Z_{CEM}
bengamide E (standard)	7.5	**250**	150	50**300**
fascaplysin (**1**)	0.4	100	**300**	200\\100
3-bromofascaplysin (**6**)	6.4	−150	0	200\\150
10-bromofascaplysin (**7**)	3.4	−100	100	200**350**
	0.8			200**300**
reticulatine (**4**)	18	150	**250**	0\\100
14-bromoreticulatine (**16**)	200	50	150	−\\−
7, 14-dibromoreticulatine (**17**)	84	−100	−100	0\\200
reticulatate (**18**)	64	50	**550**	**300**\\150
14-bromoreticulatate (**19**)	64	150		−\\−
reticulatol (**20**)	64	0		−**900**\\−**600**
14-bromoreticulatol (**21**)	84	−50	−50	−**250**\\−200

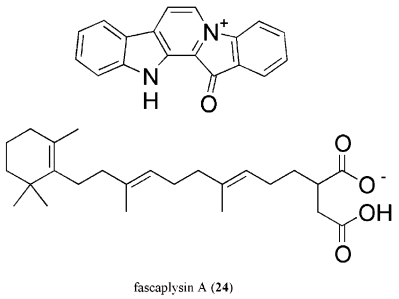
[a] Measured in zone units: 200 zone units = 6 mm. Murine cell lines: L1210 (lymphocytic leukemia), C38 (colon adenocarcinoma), CFU(GM) (colony-forming unit-granulocyte macrophage; normal hematopoietic). Human cell lines: H116 (colon), H125 (lung), CEM (leukemia), CFU(GM) (colony-forming unit-granulocyte macrophage; normal hematopoietic). [b] Significant selectivity, shown in bold, is defined by a difference of ≥250 zone units.

Scheme 1. Putative Biogenetic Pathways between Brominated Indoles, Brominated Fascaplysins, and Related Brominated Alkaloids



human leukemia, as seen in the large negative zone differentials for the human H116 versus CEM and H125 versus CEM cell lines.

Fascaplysin (**1**, NSC622398), 3-bromofascaplysin (**6**, NSC700409), and fascaplysin A (**24**, NSC660649) were



fascaplysin A (**24**)

submitted to the National Cancer Institute−Development Therapeutics Program (NCI-DTP) 60 cell line screen.[34] A subset of the data obtained is reported in Table 6 (see Table S3 for full IC_{50} set). The entries chosen for Table 6 illustrate that there can be differential sensitivities to the fascaplysins for the individual cell lines within each of the seven different tumor lines. The overall level of activity reveals that **1** and **24** are approximately equally active, but the presence of the sesterterpene anion causes activity in some cell lines where **1** is inactive. Interestingly, the presence of a bromine in **6** produces an overall increase in activity as compared to **1** and **24** but also causes inactivity in cell lines that are sensitive to **1** and **24**.

The fascaplysins and related alkaloids have consistently been isolated by our laboratory from extracts of *F. reticulata*, suggesting that these compounds might be chemical markers for this sponge species. However, the compilation of Table 1 shows that type **II** and **III** frameworks are found only in *F. reticulata*. Noteworthy is only type **I** compounds, ubiquitous in sponges, are also isolated from tunicates including the type **Ic** compounds reported above. The yields of the major metabolites such as **1** and **4**, which were observed widely in this study, were substantial. In view of recent discussions in the literature[35] this might suggest they are produced de novo by the sponge and/or tunicate, but more experiments are needed to resolve this point.[36]

In the disk diffusion soft agar colony formation assay type **Ia** compounds were the most potent, with the placement of the bromine playing a significant role. Similarly,

Table 6. Selected NCI-DTP[a] Antitumor Activity Results against in Vitro 60-Cell Line Data (IC_{50} μM)[b]

	tumor type	**1**[c] NSC 622398	**6** NSC 700409	**24** NSC 660649
NSCL	HOP-62	4.7	NA[d]	5.1
	NCI-H23	4.5	0.89	5.1
	NCI-H322M	5.0	0.65	4.3
	NCI-H522	4.5	0.68	6.3
colon	COLO-205	1.2	NA	0.83
	HCC-2998	1.7	0.91	3.8
	HCT-116	0.60	0.76	1.6
CNS	SF-295	3.9	0.59	5.2
	SNB-19	6.1	0.66	5.4
	U251	3.6	NA	4.1
melanoma	MALME-3M	0.36	0.49	0.54
	M14	0.92	0.59	4.2
	SK-MEL-5	2.0	NA	0.53
	UACC-62	4.9	0.63	3.2
ovarian	IGROV1	4.5	0.74	1.7
	OVCAR-3	1.5	4.4	3.1
	OVCAR-4	5.3	0.59	4.5
	OVCAR-8	NA	0.60	5.3
renal	RXF-393	3.5	3.1	3.5
	CAKI-1	6.1	1.6	7.3
	SN12C	4.2	2.9	3.2
	UO-31	NA	0.53	5.6
breast	HS 578T	NA	0.59	NA
	BT-549	NA	0.59	5.1

[a] Data provided by the National Cancer Institute−Development Theraputic Program (NCI-DTP). For the comprehensive data set against 60 cell lines see Table S3. [b] $IC_{50} = LC_{50}$ single-point data reported on the website http://dtp.nci.nih.gov/. [c] Fascaplysin (**1**) was submitted for testing by Prof. Chris M. Ireland, University of Utah. [d] NA (not active, $IC_{50} > 10$ μM).

bromine substitution in class **II** compounds caused a marked decrease or complete loss of both potency and selectivity. The importance of the functional group attached to the D ring in nonbrominated class **II** compounds was revealed as subtle changes demonstrated considerably different selectivities. The in vitro potency of **1**, **6**, and **24**, all of type **Ia**, in the NCI 60 cell line screen firmly demonstrates the interesting cytotoxic properties for this framework. Thus, an important follow-up has begun and involves in vivo evaluation of **24** in tumor-bearing mice to better define its pharmacological properties, and these results will be reported elsewhere.

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 500 MHz (^{1}H, MeOH-d_4, DMSO-d_6, CD_2Cl_2) and 125 MHz (^{13}C, MeOH-d_4, and DMSO-d_6). Final NMR assignments were based on comparison to previously published data and 2D NMR data derived from gHMQC, gHMBC, NOESY, and COSY. LCMS was performed with a reversed-phase 5 μm analytical column using photodiode array (PDA) and evaporative light scattering (ELS) detection with an electrospray ionization time of fight (ESITOF) mass spectrometer. Sephadex LH-20 was used for separation of the crude fractions. Preparative HPLC was performed using a single-wavelength (λ = 254 nm) UV detector and ELS detector in series. HPLC was performed with a reversed-phase 5 μm column. Silica gel 60 (230−240 mesh) was used for vacuum chromatography. FAB as well as ESITOF mass spectrometers were employed for HRMS. The optical rotation was acquired using a digital polarimeter.

Biological Material, Collection, and Identification. Specimens of *Fascaplysinopsis reticulata* were collected from Indonesia (coll. nos. 94640 and 95604) and Fiji (coll. nos. 89128 and 00104). The Indonesia specimen 94640 was collected off the coast of Pahena in the Pulau-Pulau Region, using scuba, at depths of 30−50 feet: IS#11 (N 2°40′, E 125°30′). The other Indonesia specimen, 95604, was collected from two separate sites in the Kepulauan Togian Region, using scuba, at depths of 20−100 feet: TOG#2 (S 0°29.911′, E 122°31.368′) and TOG#7 (S 0°24.737′, E 121°38.449′). The Fiji specimen 89128 was collected off the coast of Thanggalai Island (coordinates of the Island: S 17 °46.60′, E 178°43.60′). The other Fiji specimen, 00104, was collected from two separate sites, Side Streets (S 18°22.12′, E 177°58.28′) and 7 Sisters (S 18°22.25′, E 177°58.72′).

Specimens of the tunicate 115T93, *Didemnum* sp. Monniot and Monniot 1997, were collected at 15−40 feet just inside the Northeast Pass at Chuuk Atoll, Federated States of Micronesia, in August 1993 and stored in a freezer until workup. Specimens of the tunicate 10IN95, *Didemnum* sp. Monniot and Monniot 1997, were collected in Northern Sulawesi, Indonesia, in October 1995 and preserved in alcohol in a cold room until workup.

Fascaplysinopsis reticulata (UCSC coll. no. 89128, 94640, 95604, and 00104):[37] the specimens were subglobular to lobate in shape, with a characteristic thick skin (unarmored dermis) and large widely spaced conules (2−4 mm high, up to 1 cm apart). Their external color was of intense reddish-brown and greenish in color, while the interior was brown-orangish. The sponge is firm but compressible. Specimen 95604 had one oscule on its top. The skeleton presents a system of cored primary fibers (with foreign debris), strongly fascicular throughout the sponge, and uncored secondary fibers that form an irregular branching network.

Didemnum sp. (OU coll. no. 115T93 and 10IN95): both specimens were colonial, relatively small, and elongated with a brownish-purple color underwater (see Figures S29 and S30 for underwater photographs). As no larvae were observed, the species of either specimen could not be identified.

Extraction and Isolation. The sponges were preserved according to our standard procedure as described previously[32a] and then transported to the home laboratory at ambient temperature. Each collection was worked up separately. The organisms were soaked three successive times for 24 h in 100% MeOH. The sponge was additionally soaked two successive times for 24 h in 100% CH_2Cl_2. The resulting oil was partitioned as described elsewhere.[27]

Pure compounds were obtained as follows. A 2 g portion of the FD extract of *F. reticulata* (Indonesia, coll. no. 94640) was fractionated using Sephadex LH-20 with 100% MeOH. The third Sephadex fraction (733.5 mg) was separated using HPLC with a gradient of 70:30 up to 50:50 H_2O−MeOH (0.1% formic acid in both solvents) to yield **1** (64.0 mg), **6** (10.1 mg), and **7** (10.1 mg).

A 1 g portion of the DMM extract of *F. reticulata* (Indonesia, coll. no. 95604) was fractionated using Sephadex LH-20 with 100% MeOH. The fifth Sephadex fraction (85 mg) was separated using HPLC with a gradient of 85:15 to 80:20 H_2O−CH_3-CN (0.1% formic acid in both solvents) to yield **3** (15.7 mg) and **16** (21.3 mg). A 400 mg portion of the FM extract was similarly fractionated using Sephadex LH-20, and the second and third Sephadex fractions were subjected to HPLC with a gradient of 25:75 to 65:35 MeOH−H_2O (0.1% formic acid in both solvents). Pure compounds from each were combined to yield **19** (3.5 mg). A separate 2 g portion of the DMM extract of 95604 was fractionated using Sephadex LH-20 with 100% MeOH. The fourth Sephadex fraction (581.4 mg) was separated using HPLC with a gradient of 70:30 to 40:60 H_2O−MeOH (0.1% formic acid in both solvents) to yield six fractions. The fifth fraction (24.5 mg) was further purified by HPLC to give **8** (1.4 mg) and **17** (4.2 mg). A different 2 g portion of the DMM extract was similarly fractionated using Sephadex LH-20, and the seventh fraction was subjected to HPLC with a gradient of 25:75 to 65:35 MeOH−H_2O (0.1% formic acid in both solvents) to yield **21** (3.5 mg). The last Sephadex fractions from four separate runs of 95604 DMM were combined to give 682.5 mg. This was subsequently separated by preparative HPLC to yield 21 fractions. Fractions 13 and 10 were further purified by semipreparative HPLC [52:48 to 82:18 ACN−H_2O (0.1% TFA added to both solvents)] for fraction 13 and fraction 10

[42:58 to 72:28 ACN−H_2O (0.1% TFA added to both solvents)] to give **22** (12.3 mg) and **23** (6.6 mg), respectively.

A 500 mg portion of the DMM extract of *F. reticulata* (Fiji, coll. no. 89128) was fractionated using Sephadex LH-20 with 100% MeOH. The fifth and sixth Sephadex fractions were separated using HPLC with a gradient of 90:10 to 50:50 H_2O−MeOH (0.1% formic acid in both solvents). Pure compounds from each were combined to yield **18** (2.5 mg). A separate 2 g portion of the DMM extract of 89128 was fractionated using Sephadex LH-20 with 100% MeOH. The fifth fraction was separated using HPLC with a gradient of H_2O−MeOH (0.1% formic acid in both solvents) to afford **20** (1.7 mg). A 1 g portion of the FD extract of *F. reticulata* (Fiji, coll. no. 00104) was fractionated using preparative HPLC with a gradient of 20:80 up to 50:50 ACN−H_2O (0.1% TFA added to both solvents) to yield nine fractions. The first and second fractions were separated using HPLC with a gradient of 20:80 up to 40:60 ACN−H_2O (0.1% TFA added to both solvents) to afford **9** (2.7 mg). The third and sixth preparative fractions supplied **1** (50 mg) and **4** (31.7 mg), respectively.

Specimens of *Didemnum* sp. (115T93) were cut into small pieces and soaked in MeOH followed by MeOH−CH_2Cl_2 (1:1). The extracts were concentrated, combined, and partitioned between aqueous MeOH and organic solvents as described previously[38] to yield, after evaporation of solvents in vacuo, hexane, CH_2Cl_2, *n*-BuOH, and H_2O fractions. The CH_2Cl_2 fraction was fractionated on a silica gel open column using increasing amounts of MeOH in CH_2Cl_2 as eluent (10−25% MeOH−CH_2Cl_2). The major component, fascaplysin (**1**) (82.4 mg), was obtained from a more polar fraction by reversed-phase HPLC using 50% H_2O−MeOH containing 0.1% TFA as eluent. Similar treatment of a less polar fraction yielded 3-bromofascaplysin (**6**) (9.5 mg).

The *Didemnum* sp. tunicate (10IN95) samples were immersed in 50% H_2O−EtOH immediately after collection. After soaking 1 day, the aqueous alcohol was decanted and the wet sample shipped to OU, where it was stored in MeOH in a cold room. Later the MeOH was decanted and the specimens were extracted again with MeOH and then twice with CH_2Cl_2. The extracts were concentrated, combined, and partitioned between aqueous MeOH and organic solvents as described previously[38] to yield, after evaporation of solvents in vacuo, hexane, CH_2Cl_2, *n*-BuOH, and H_2O fractions. The CH_2Cl_2 fraction was subjected to flash chromatography over silica gel using a step gradient of CH_2Cl_2 with increasing concentrations of MeOH to yield 11 fractions. Fractions 1, 5, and 8 were purified by repeated reversed-phase HPLC using 15% H_2O−MeOH, 50% H_2O−MeOH containing 0.01% TFA, and 70% H_2O−MeOH, respectively, as eluents. Purification of fraction 1 afforded, in order of elution, compounds **11** (0.7 mg), **14** (1.0 mg), **12** (0.3 mg), **13** (0.3 mg), and **15** (0.6 mg). Fraction 5 yielded **1** (7.0 mg) and **6** (2.0 mg), while fraction 8 provided **9** (3.7 mg), which was also isolated from the butanol fraction together with **1** and tryptamine.

Disk Diffusion Soft Agar Colony Formation Assay. An in vitro cell-based assay was employed to identify solid tumor selectivity for pure compounds. The differential cytotoxicity[32b] is expressed by observing a zone differential of 250 units or greater between any solid tumor cell (Colon38, ColonH116, LungH125) and either leukemia cells (L1210 or CEM) or normal cells (CFU-GM). Results are designated as "tumor selective" (zone units of solid tumor − normal cells) or "solid tumor selective" (zone units of solid tumor − leukemia tumor). The activity results appear in Tables 5 and S1.

Fascaplysin (1): red solid; LRESITOFMS *m*/z 271 $[M]^+$; ^1H and ^{13}C NMR (MeOH-d_4) data were in agreement with literature values.[1]

Reticulatine (4): yellow solid; LRESITOFMS *m*/z 303 $[M]^+$; ^1H and ^{13}C NMR (MeOH-d_4) data were in agreement with literature values.[11]

3-Bromofascaplysin (6): red solid; HRESITOFMS *m*/z 348.9971 $[M]^+$, Δ 2.4 mmu of calcd; ^1H NMR (500 MHz) in Figure S1 and Table 1 of ref 9; ^{13}C NMR (125 MHz) in Figure S2 and Table 1 of ref 9.

10-Bromofascaplysin (7): red solid; HRESITOFMS *m*/z 349.0004 $[M]^+$, Δ 3.3 mmu of calcd; ^1H NMR (500 MHz) in Figure S3 and Table 3; ^{13}C NMR (125 MHz) in Figure S4 and Table 3.

3,10-Dibromofascaplysin (8): red solid; HRESITOFMS *m*/z 426.9007 $[M]^+$, Δ 6.9 mmu of calcd; ^1H NMR (500 MHz) in Figure S5 and Table 3; ^{13}C NMR (125 MHz) in Figure S6 and Table 3.

Homofascaplysate A (9): yellow solid; $[\alpha]^{25}_D$ +14° (*c* 0.25, MeOH); HRFABMS *m*/z 331.1101 $[M]^+$, Δ 2.3 mmu of calcd; ^1H NMR (500 MHz) in Figure S7 and Table 3; ^{13}C NMR (125 MHz) in Figure S8 and Table 3.

Homofascaplysin B-1 (11): red oil; FABMS *m*/z 357 $[M + H]^+$, 283 [M − C(O)OEt], 255 [M − C(O)C(O)OEt]; ^1H NMR (500 MHz) (assignments based on COSY data) in Figure S9 and Table 3.

3-Bromohomofascaplysin B (12): yellow solid; FABMS *m*/z 421/423 (1:1) $[M + H]^+$, 361/363 (1:1) [M − $C(O)CH_3$]; HRFABMS *m*/z 421.0165 $[M + H]^+$, Δ 2.3 mmu of calc; ^1H NMR (500 MHz) (assignments based on COSY data) in Figure S10 and Table 3.

3-Bromohomofascaplysin B-1 (13): yellow solid; FABMS *m*/z 435/437 (1:1) $[M + H]^+$, 361/363 (1:1) [M − C(O)OEt]; HRFABMS *m*/z 435.0348 [M + H], Δ 0.4 mmu of calcd; ^1H NMR (500 MHz) (assignments based on COSY data) in Figure S11 and Table 3.

Homofascaplysin C (14): yellow solid; FABMS *m*/z 285 $[M + H]^+$; ^1H and ^{13}C NMR data were in agreement with literature values.[10]

3-Bromohomofascaplysin C (15): yellow solid; ESIMS *m*/z 363/365 (1:1) $[M + H]^+$; ^1H NMR (500 MHz) (assignments based on COSY data) in Figure S12 and Table 3.

14-Bromoreticulatine (16): yellow solid; HRESITOFMS *m*/z 381.0221 $[M]^+$, Δ 1.3 mmu of calcd; ^1H NMR (500 MHz) in Figure S13 and Table 1 of ref 9; ^{13}C NMR (125 MHz) in Figure S14 and Table 1 of ref 9.

7,14-Dibromoreticulatine (17): yellow solid; HRESITOFMS *m*/z 458.9353 $[M]^+$, Δ 1.5 mmu of calcd; ^1H NMR (500 MHz) in Figure S15 and Table 4; ^{13}C NMR (125 MHz) in Figure S16 and Table 4.

Reticulate (18): yellow solid; HRESITOFMS *m*/z 289.0972 $[M]^+$, Δ 0.1 mmu of calcd; ^1H NMR (500 MHz) in Figure S17 and Table 1 of ref 9; ^{13}C NMR (125 MHz) in Figure S18 and Table 1 of ref 9.

14-Bromoreticulate (19): yellow solid; HRESITOFMS *m*/z 367.0096 $[M]^+$, Δ 1.9 mmu of calcd; ^1H NMR (500 MHz) in Figure S19 and Table 1 of ref 9; ^{13}C NMR (125 MHz) in Figure S20 and Table 1 of ref 9.

Reticulatol (20): yellow solid; HRESITOFMS *m*/z 261.1041 $[M]^+$, Δ 1.9 mmu of calcd; ^1H NMR (500 MHz) in Figure S21 and Table 4; ^{13}C NMR (125 MHz) in Figure S22 and Table 4.

14-Bromoreticulatol (21): yellow solid; HRESITOFMS *m*/z 339.0085 $[M]^+$, Δ 4.2 mmu of calcd; ^1H NMR (500 MHz) in Figure S23 and Table 4; ^{13}C NMR (125 MHz) in Figure S24 and Table 4.

3-Bromosecofascaplysin A (22): yellow solid; HRESITOFMS *m*/z 397.0160 $[M + H]^+$, Δ 2.2 mmu of calcd; ^1H NMR (500 MHz) in Figure S25 and Table 4; ^{13}C NMR (125 MHz) in Figure S26 and Table 4.

3-Bromosecofascaplysin B (23): yellow solid; HRESITOFMS *m*/z 383.0102 $[M + H]^+$, Δ 7.7 mmu of calcd; ^1H NMR (500 MHz) in Figure S27 and Table 4; ^{13}C NMR (125 MHz) in Figure S28 and Table 4.

Acknowledgment. The work at UCSC was supported by the National Institutes of Health (R01 CA 47135 and U19 CA 52955). Additional financial support at UCSC was from equipment grants from NSF BIR-94-19409 (NMR) and a supplement to NIH CA52955 (ESITOFMS). The work at OU was supported by NIH grant CA 52955. S.J.R. received support from the National Science Foundation (REU CHE-9987824). Sponge taxonomy analyses were generously provided by M. C. Diaz (UCSC). We thank the government of Chuuk State, Federated States of Micronesia, for allowing us to collect

tunicate specimen 115T93 and the Coral Reef Research Foundation for collaboration and assistance in specimen collection. Tunicate taxonomy analyses were generously provided by F. Monniot of the Museum National d'Histoire Naturelle, Paris, France. We thank S. Lopez (UCSC) for help with the isolation of new compounds and T. A. Johnson (UCSC) for supplying **6** for the disk diffusion assay.

Supporting Information Available: ^{1}H and ^{13}C NMR spectral data of **6**−**9**, **16**−**23**; ^{1}H NMR spectral data of **11**−**13**, **15**; underwater photos of tunicate specimens, comparison NMR data for **20** vs **4** and **21** vs **16**, full zone unit data in the disk diffusion soft agar colony formation assay, and complete IC_{50} data from the NCI 60 cell line panel. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP049935+