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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2013

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Additional Cytotoxic Pyridoacridine Alkaloids from the Ascidian *Cystodytes violatinctus* and Biogenetic Considerations

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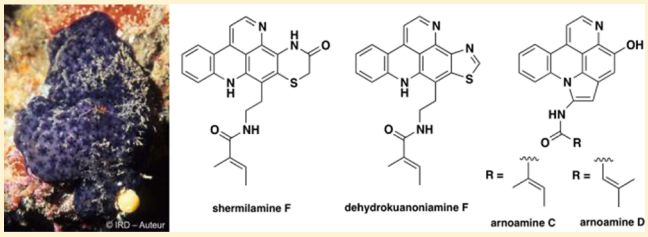
Supporting Information

ABSTRACT: The extraction and purification of the bioactive extract of *Cystodytes violatinctus* (Solomon Islands) led to the isolation and identification of six pyridoacridine alkaloids. The structures of four new members of this family, shermilamine F (**1**), dehydrokuanoniamine F (**2**), and arnoamines C (**3**) and D (**4**), were elucidated on the basis of NMR and MS data and by comparison with data of known compounds isolated from this genus. A general hypothetical biogenetic pathway is then proposed for pyridoacridine alkaloids that contain a fused pyrrole ring. Comparison of the biological properties of the isolated alkaloids is also discussed.



A large variety of pyridoacridine alkaloids, known to show *in vitro* cytotoxicity against cultured tumor cell lines, have been isolated from ascidians of the genus *Cystodytes*. In previous studies, we described two major chemotypes within the most abundant color morphs of *Cystodytes dellechiajei* (Della Valle, 1877) in the Northwestern Mediterranean,[1] and we also characterized new members of this structure family.[2,3] Our ongoing project to investigate different specimens of the purple color morph of *Cystodytes* spp. collected in different marine areas worldwide led to the isolation of a series of pyridoacridine alkaloids from the ascidian *Cystodytes violatinctus* (Monniot, 1988), collected in the South-Pacific Ocean. This paper presents the isolation, structure elucidation, and biological activity of four new pyridoacridine alkaloids, shermilamine F (**1**), dehydrokuanoniamine F (**2**), and arnoamines C (**3**) and D (**4**), along with the known shermilamine C (**5**) and dehydrokuanoniamine B (**6**) previously reported from a Fijian *Cystodytes* sp.[4] These results inspired the construction of a general biogenetic pathway for pyridoacridines with a pyrrole ring.

Pyridoacridines are a class of strictly marine-derived alkaloids. Since the publication of the first two members of this family, calliactine[5] in 1940 and amphimedine in 1983,[6] the family has grown. In the review published by Molinski in 1993,[7] the number of pyridoacridine alkaloids was estimated to be 40, while today more than 80 are known (MarinLit database, 2012). Different graphical representations, lettering of rings, and numbering have been adopted in the literature for these compounds. This can make comparisons of chemical shifts for specific atoms between molecules, or discussions of biogenetic considerations, highly difficult. As the structures of these polycyclic aromatic alkaloids are based on the 7*H*-pyrido[4,3,2-*mn*]acridine skeleton, we propose to adopt, according to IUPAC, a graphical representation in which the tricyclic acridine system is horizontally oriented (A−B−C ring system) with the fused pyridine ring (D) on the top right of the B−C rings. The parent tetracyclic system thus oriented is numbered as shown in Figure 1 with the additional E ring, if present, primed. With this numbering, atoms of the A−B−C−D rings system in styelsamine-, kuanoniamine-, or shermilamine-type pyridoacridines will always keep the same

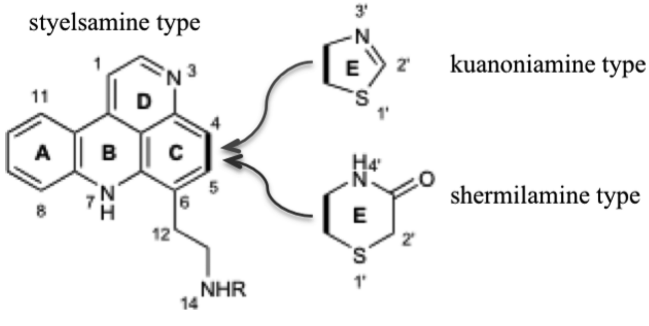


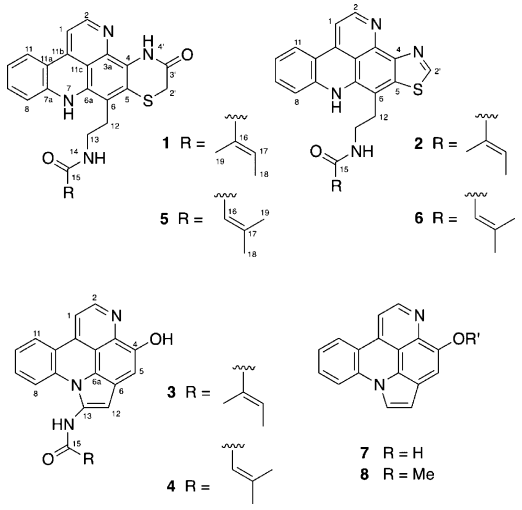
Figure 1. Proposed numbering of pyridoacridine alkaloids.

Received: April 4, 2013

dx.doi.org/10.1021/np400284z | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

number. For example, the carbon atom bearing the alkylamine side chain remains numbered C-6 whatever the presence and the structure of the additional E ring.

Specimens of *C. violatinctus* were collected off the coast of the Solomon Islands in 2006. After collection, the ascidians were immediately frozen and lyophilized. Freeze-dried material was then extracted with a mixture of CH_2Cl_2/MeOH (1:1). The extract exhibited significant cytotoxicity against the KB cell line. The residue was then fractionated by C_8 reversed-phase flash chromatography and C_{18} reversed-phase HPLC to yield the new compounds **1** (6.7 mg), **2** (3.4 mg), **3** (5.9 mg), and **4** (5.7 mg) and the known compounds **5** (4.3 mg) and **6** (4.5 mg).



All the alkaloids were purified as TFA salts. Inspection of the 1D and 2D NMR data obtained mainly by COSY, HSQC, and HMBC experiments led to the structure elucidation of the four new compounds **1**–**4** (Tables 1 and 2).

Shermilamine F (**1**) was obtained as a purple oil with the molecular formula $C_{24}H_{22}N_4O_2S$ based on (+)-HRESIMS data. An identical molecular formula was obtained for compound **5**, which was identified as shermilamine C by comparison with spectroscopic data reported in the literature.[4] Comparison of ^{1}H and ^{13}C NMR data obtained for compounds **1** (Table 1) and **5** confirmed the close similarity between these two alkaloids except for the unsaturated acyl side chain (C-16 to C-19). The signals corresponding to two vinylic methyl groups, one at δ_H 1.87 (d, J = 7.0 Hz, H_3-18) COSY correlated to a vinylic proton at δ_H 6.78 (q, J = 7.0 Hz, H-17) and a second one at δ_H 1.97 (s, H_3-19) attached to the quaternary sp^2 carbon at δ_C 130.1 (C-19), indicated the presence of a tigloyl terminus instead of the isobutenoyl found in shermilamine C (**5**). The upfield shift of the H_3-18 protons at δ_H 1.87 relative to H_3-19 (δ_H 1.97) and the upfield shift of C-19 (δ_C 12.2) relative to C-18 (δ_C 14.2) indicated that the double-bond configuration could be assigned as *E* on the basis of reported NMR data for cystodytins B, E, G, and I[8] and lissoclin B.[9] NOESY correlations between the H_3-18 and H_3-19 protons could not be obtained to confirm this geometry, due to their close chemical shifts (1.87 vs 1.97 ppm). The overall structure of **1** was finally confirmed by HMBC correlations.

Dehydrokuanoniamine F (**2**) was isolated as a purple oil, and its molecular formula was determined to be $C_{23}H_{20}N_4OS$ based on (+)-HRESIMS data. An identical molecular formula was obtained for compound **6**, identified as dehydrokuanoniamine B by comparison with spectroscopic data reported in the literature.[4] NMR data (Table 1) also supported a close relationship between **2** and **6**, with the greatest differences occurring in the regions of the spectra corresponding to the acyl side chain. The structure of the side chain was supported by the presence of two vinylic methyl groups (H_3-18 and H_3-19) and a vinylic proton (H-17) exhibiting the same coupling pattern of homo- and heteronuclear correlations as already observed in compound **1**. As previously mentioned for compound **1**, the relative values of chemical shifts for H_3-18 and H_3-19 of compound **2** (Table 1) allowed the assignment of the configuration of the side chain double bond as *E*.

Arnoamines C (**3**) and D (**4**) were isolated as orange oils, and accurate mass measurement of the parent ions by HRESIMS provided the common formula $C_{22}H_{18}N_3O_2$ for both compounds. The UV spectra of the TFA salts of **3** and **4** revealed the same absorption maxima (λ_{max} = 275, 278, 400, and 477 nm). Comparison of ^{1}H and ^{13}C NMR data obtained for compounds **3** and **4** confirmed a close structural relationship.[10] Analysis of ^{1}H NMR, HSQC, and COSY data of compound **3** (Table 2) established a tetracyclic pyridoacridine moiety comprising three characteristic aromatic spin systems: an *ortho*-disubstituted benzene ring (H-8 to H-11), a 2,3,4-trisubstituted pyridine ring (H-1 and H-2), and an isolated aromatic proton (H-5). Heteronuclear correlations of the singlet at δ_H 7.93 (H-5) with the two quaternary carbon atoms at δ_C 130.0 (C-3) and 143.9 (C-4) and a more shielded one at δ_C 117.5 (C-6) placed H-5 on an isolated 2,3,4,5-tetrasubstituted phenol. The phenolic moiety was confirmed by the presence of an additional exchangeable proton signal at δ_H 11.06 (OH-4). Such a reduced iminoquinone substructure has been previously described in the arnoamines[11] and styelsamines,[12] and comparison of NMR data allowed the assignment of the remaining proton and carbon signals of this moiety. Indeed, the remaining aromatic signal was a singlet at δ_H 7.21 (H-12) showing HMBC correlations with the three quaternary carbon atoms at δ_C 113.1 (C-6a), 117.5 (C-6), and 131.2 (C-13). Moreover, the HMBC correlation between H-5 and C-12 placed H-12 on a pyrrole ring substituted at C-13 and fused to the tetracyclic pyridoacridine moiety as previously described in cycloshermilamine D[13] and stelletamine.[14] The remaining signals included two allylic methyl groups [δ_H 1.91 (s, H_3-18) and 1.98 (q, J = 6.9 Hz, H_3-19)], an allylic coupled proton [δ_H 6.84 (q, J = 6.9 Hz, H-17)], and an amide carbonyl [δ_C 169.5 (C-15), δ_H 10.57 (NH-14)] that were reminiscent of the tigloyl group found in compounds **1** and **2** (Table 2) and connected to the pyrrole ring at the C-13 quaternary sp^2 carbon atom (δ_C 131.4). The proposed *E* configuration of the double bond, based upon similarities with data previously obtained for compounds **1** and **2**, led to structure **3**. Inspection of the ^{1}H and ^{13}C NMR data for compound **4** clearly showed that the only difference between both compounds lies in the position of the methyl groups attached to the double bond of the side chain (Table 2). The multiplicities of the signals at δ_H 1.99 (3H, br s) and 2.17 (3H, s), assigned to respectively H_3-18 and H_3-19, and COSY correlations between H_3-18 and H-16 at δ_H 6.20 (1H, br s) were consistent with a 3-methylcrotonoylamino at C-13, as a cross-peak between H-16 and C-15 was observed in the HMBC spectrum. The upfield shift of C-18 suggested that H-16 and H-18 were in a *cis* relative configuration.[8,15] The structure of the side chain was also confirmed by comparison with the corresponding NMR data obtained for compounds **5** and **6**. The new compounds were named arnoamines C (**3**) and D (**4**), as they are structurally related to arnoamines A (**7**) and B (**8**) previously isolated from the ascidian *Cystodytes* sp. collected in the Marshall Islands.[9] They are the first examples of pyrrole-containing

dx.doi.org/10.1021/np400284z | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. NMR Spectroscopic Data of the TFA Salt of 1 in $CDCl_3$ and the TFA Salt of 2 in DMSO-d_6

	shermilamine F (**1**)			dehydrokuanoniamine F (**2**)		
position	δ_C, type[a]	δ_H (J in Hz)[b]	HMBC[c]	δ_C, type[a]	δ_H (J in Hz)[b]	HMBC[c]
1	104.9, CH	7.29, d (6.2)	2, 11a, 11c	107.9, CH	7.85, m[e]	
2	144.6, CH	8.41, d (6.2)	1, 3a, 11b	144.8, CH	8.55, m[e]	
3a	129.3, C			nd		
4	118.2, C			135.3, C[d]		
5	130.1, C			144.0, C[d]		
6	111.7, C[d]			107.4, C		
6a	132.2, C			133.0, C		
7		11.87, br s			11.24, br s	
7a	140.8, C			140.3, C		
8	118.5, CH	7.85,d 8.3)	10, 11a	117.3, CH	7.68, m	7a, 10
9	134.6, CH	7.66, dd, (8.1, 8.3)	7a, 8, 11	134.4, CH	7.68, m	7a, 10, 11
10	123.0, CH	7.26, dd, (8.1, 8.3)	8, 11a	122.5, CH	7.23, m	8, 9, 11a
11	124.3, CH	7.96, d (8, 1)	7a, 11b	127.1, CH	8.26, d (8.4)	
11a	115.0, C			114.6, C		
11b	147.0, C			152.4, C		
11c	118.5, C			118.2, C		
12	28.3, CH_2	3.21, m	5, 6, 6a, 13	31.0, CH_2	3.15, t (7.1)	5, 6, 6a
13	38.0, CH_2	3.37, m	12, 15	36.8, CH_2	3.39[f]	6, 15
14		6.70, t (7.2)	15		8.30, t (5.1)	15
15	171.5, C			169.6, C		
16	130.1, C			131.0, C		
17	133.7, CH	6.78, q (7.0)	15, 16, 18, 19	130.7, CH	6.38, q (6.2)	15, 16, 18, 19
18	14.2, CH_3	1.87, d (7.0)	16, 17	13.7, CH_3	1.68, d (6.2)	16, 17
19	12.2, CH_3	1.97, s	15, 16, 17	12.4, CH_3	1.72, s	15, 16
2′	30.2, CH_2	3.45, s	5, 3′	151.0, CH[e]	9.35, s	4
3′	164.1, C					
4′		10.33, br s				

[a]500 MHz. [b]125 MHz. [c]HMBC correlations are from proton(s) stated to the indicated carbon. [d]Not visible but assigned on the basis of $^1H-^{13}C$ direct correlations and $^1H-^{13}C$ *long-range* correlations. [e]The signals are observable as doublets (J = 6.2 Hz) in DMSO-d_6 at 60 °C. [f]Overlapped by the water signal but assigned on the basis of $^1H-^{13}C$ direct correlations.

pyridoacridines that possess a modified acrylamide side chain as a substituent on the pyrrole ring.

From a biogenetic point of view, Plubrukarn and Davidson[11] suggested that the pyrrole ring in arnoamines A and B could be formed through C-13/N-7 cyclization of the amidoethyl side chain observed in cystodytin A, an oxidized and *N*-acylated analogue of styelsamine D (**9**).[15] Four years later, Skyler and Heathcock[16] proposed that arnoamine A (**7**) and styelsamine D (**9**) can be related through hypothetical *N*-methylated intermediates and a final quaternary ammonium ion that could serve as a leaving group in the key cyclization step. Our discovery of **3** and **4** has led us to propose a slight modification (Figure 2) of the general biogenetic pathway for pyridoacridines that is based on the works of Skyler[16] and ourselves.[3]

An *acylation pathway* on the primary amine styelsamine D (**9**) can give rise to the corresponding diversity of *N*-acylated derivatives. From a synthetic point of view, amide oxidation into an acyliminium can occur under several conditions (light, aerobic oxidation, or electrochemistry),[17−19] and some enzymes have also been shown to catalyze this reaction.[20] The high nucleophilicity of the acridine nitrogen could give rise to a unique cyclization with the resulting acyliminium at this position. Finally, a spontaneous oxidative process toward a totally conjugated system may lead to the formation of the new compounds **3** and **4** (Figure 2).

This proposition could explain the biogenesis of the arnoamines found in purple *C. violatinctus*. Of course all of these steps require clear confirmation by *in vivo* studies or cell-free extracts, and this work is ongoing.

The 50% inhibitory concentrations (IC_{50}) of the six isolated compounds were evaluated in A375 (melanoma) and HCT116 and SW480 (colon) cancer cell lines, using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test (Table 3). The well-known cytotoxic pyridoacridine ascididemin[21] used here as a positive control displayed strong cytotoxic activity in all cell lines (IC_{50} < 1 μM). Among the newly discovered compounds, arnoamine D (**4**) appeared as the most active, with micromolar IC_{50} values in all cell lines tested. Dehydrokuanoniamine F (**2**) showed good activity selective to the SW480 cell line. All other compounds displayed a similarly low activity against all three cell lines. As already demonstrated, the cytotoxicity of ascididemin is mainly due to its ability to intercalate into DNA due to its planar pentacyclic chromophore.[21] We could hypothesize that the relative low activity of the new compounds is due to the presence of the nonplanar side chain in all of the structures.

In summary, four new pyridoacridine alkaloids have been isolated from the ascidian *C. violatinctus* collected in the South-Pacific Ocean. Arnoamines C and D are particularly intriguing due to the presence of a modified acrylamide side chain as a substituent on the pyrrole ring.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Hewlett-Packard diode array spectrophotometer. An FT-IR Bruker

dx.doi.org/10.1021/np400284z | J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. NMR Spectroscopic Data for the TFA Salts of 3 and 4 in DMSO-d_6

	arnoamine C (3)				arnoamine D (4)			
position	δ_C, type[a]	δ_H[b]	δ_H (J in Hz)[c]	HMBC[d]	δ_C, type[a]	δ_H[b]	δ_H (J in Hz)[c]	HMBC[d]
1	110.6, CH	8.60	8.63, d (5.9)		110.6, CH	8.56	8.74, d (6.4)	2, 11c
2	141.6, CH	9.03	9.06, d (5.9)		140.5, CH	8.95	9.05, d (6.4)	1, 11b
3a	130.0, C				nd			
4	143.9, C				142.9, C			
5	109.1, CH	7.93	7.93, s	4, 6, 6a	110.2, CH	7.95	8.08, br s	4, 6
6	117.5, C[e]				117.1, C			
6a	113.1, C[e]				118.0, C[e]			
7a	136.0, C				136.0, C			
8	116.6, CH	8.53	8.56, d (7.9)	7a, 10, 11a	116.7, CH	8.56	8.70, d (8.5)	10, 11a
9	133.2, CH	7.97	7.97, dd (7.9, 7.8)	7a, 11	133.8, CH	7.95	8.06, dd (8.5, 8.4)	8, 11
10	124.7, CH	7.69	7.71, dd (7.9, 7.8)	11a	125.0, CH	7.65	7.76, dd (8.4, 8.5)	11a
11	127.0, CH	8.91	8.96, d (7.8)	7a, 9	127.3, CH	8.84	9.02, d (8.4)	7a, 9
11a	119.0, C				118.7, C			
11b	nd				138.6, C			
11c	116.4, C				113.0, C			
12	106.2, CH	7.21	7.22, s	6, 6a, 13	106.3, CH	7.24	7.32, s	6, 13
13	131.4, C				131.4, C			
14		10.57	10.51, s	15		10.73	10.48, br s	
15	169.5, C				169.6, C			
16	131.2, C				117.6, CH	6.20	6.17, br s	15, 18, 19
17	133.0, CH	6.84	6.83, q (6.9)	15, 18, 19	154.5, C			
18	14.0, CH_3	1.91	1.89, d (6.9)	16, 17	27.4, CH_3	1.99	1.97, br s	16, 17, 19
19	12.3, CH_3	1.98	1.95, s	15, 17	19.8, CH_3	2.17	2.15, s	16, 17, 18
OH (4)		11.06				10.30		

[a]125 MHz. [b]500 MHz. [c]400 MHz at 60 °C. [d]HMBC correlations are from proton(s) stated to the indicated carbon. [e]Interchangeable.

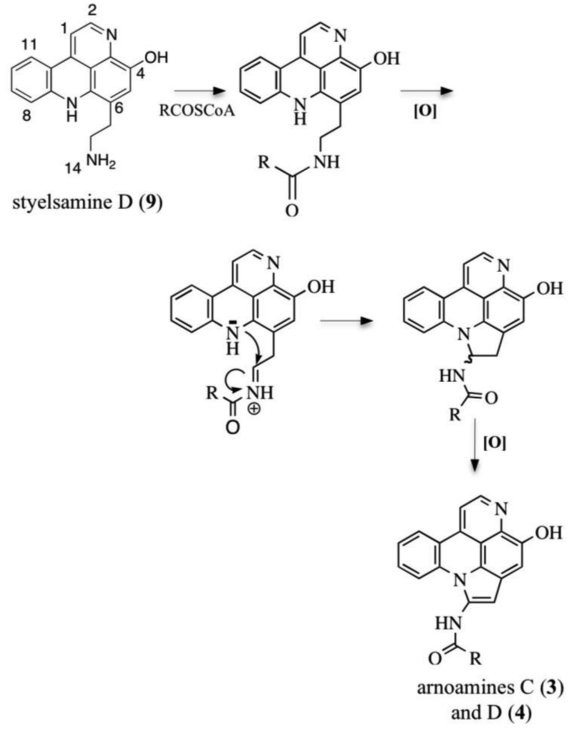


Figure 2. Proposed biogenetic acylation pathway connecting styelsamine D (**9**) to arnoamines C (**3**) and D (**4**). RCOSCoA = acyl-coenzyme A.

Tensor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were acquired at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Jeol EX 400 spectrometer and at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Bruker Advance II spectrometer equipped with a cryoprobe. 2D

Table 3. IC_{50} Values (in μM) of Reference and Test Compounds in the HCT116, SW480, and A375 Cell Lines[a]

	HCT116	SW480	A375
ascididemin	0.25	0.10	0.38
	0.11−0.57	0.07−0.14	0.21−0.67
shermilamine F (**1**)	>10	>10	>10
dehydrokuanoniamine F (**2**)	>10	3.30	>10
		1.40−7.78	
arnoamine C (**3**)	18.90	>10	>10
	2.26−8.25		
arnoamine D (**4**)	4.32	8.48	6.00
	7.88−45.90	4.42−16.30	2.47−14.50
shermilamine C (**5**)	19.00	9.99	>10
	7.88−45.90	3.12−32.00	
dehydrokuanoniamine B (**6**)	14.40	8.02	11.8
	5.54−37.40	4.64−13.80	4.32−32.00

[a]The second line for each cell type shows the 95% confidence interval for IC_{50}. ">10" means that the sigmoidal dose−response curve could not be fitted due to low activity of the compound.

experiments were performed using Jeol and Bruker standard pulse programs, respectively. LRESIMS and HRESIMS were recorded on a Waters LCT TOF ESI spectrometer. Vacuum column chromatography was performed on Merck Lichroprep RP-8 (0.04-0.063 mm). Preparative HPLC was carried out using binary Waters 1525 pumps and a Waters 2487 dual λ absorbance detector on an Interchrom Uptisphere 5 μm, 250 × 10 mm, ODB column.

Biological Material. Specimens of *Cystodytes violatinctus* (Monniot, 1988) (order Aplousobranchia, family Polycitoridae), a dark purple ascidian, were collected off the coast of the Solomon Islands (−15 m) in 2006 (GPS position 11°27.868′ S, 160°00.76′ E). A voucher specimen is available under the accession number UA435 (CEAB, Spain).

dx.doi.org/10.1021/np400284z | J. Nat. Prod. XXXX, XXX, XXX−XXX

Collection, Extraction, and Isolation Procedures. After collection, the ascidians were immediately frozen, lyophilized, and kept frozen until used. Freeze-dried material (18 g) was then extracted three times with a mixture of CH_2Cl_2/MeOH (1:1). The extract was concentrated to yield an orange oil (1.9 g). The residue was then fractionated by reversed-phase C_8 flash chromatography with increasing amounts of MeOH in H_2O. Fractions containing alkaloids, eluted with 50% and 80% MeOH, were further purified by HPLC on a C_{18} reversed-phase column eluting with an appropriate MeOH/aqueous 0.1% trifluoroacetic acid gradient. HPLC purification of the less polar fraction yielded compounds **1** (6.7 mg), **2** (3.4 mg), **5** (4.3 mg), and **6** (4.5 mg), while the most polar one yielded compounds **3** (5.9 mg) and **4** (5.7 mg).

Shermilamine F (1): purple oil; UV (MeOH, H^+) λ_{max} (log ε) 230 (4.2), 278 (3.9), 301 (4.1), 317.7 (4.3), 361 (3.4), 381 (3.4), 524 (3.3) nm; IR (film) 3297, 3061, 2922, 1642, 1536, 1479 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 431 $[M + H]^+$; HRESIMS m/z 431.1529 $[M + H]^+$ (calcd for $C_{24}H_{22}N_4O_2S$ 431.1542).

Dehydrokuanoniamine F (2): purple oil; UV (MeOH, H^+) λ_{max} (log ε) 229 (4.0), 268 (3.9), 307 (4.2), 317.7 (4.3), 358 (4.3), 528 (3.2) nm; IR (film) 3310, 3030, 2921, 1653, 1424 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 401 $[M + H]^+$; HRESIMS m/z 401.1448 $[M + H]^+$ (calcd for $C_{23}H_{20}N_4OS$, 401.1436).

Arnoamine C (3): orange oil; UV (MeOH, H^+) λ_{max} (log ε) 275 (4.0), 278 (4.0), 310 (3.8), 402 (3.4), 473 (3.4) nm; IR (film) 3442, 3232, 2923, 1664, 1559, 1490, 1450 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS m/z 356 $[M + H]^+$; HRESIMS m/z 356.1398 $[M + H]^+$ (calcd for $C_{22}H_{18}N_3O_2$, 356.1399).

Arnoamine D (4): orange oil; UV (MeOH, H^+) λ_{max} (log ε) 275 (4.0), 277 (4.0), 309 (3.8), 403 (3.4), 473 (3.4) nm; IR (film) 3440, 3235, 2922, 2915, 1665, 1559, 1491, 1449 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS m/z 356 $[M + H]^+$; HRESIMS m/z 356.1402 $[M + H]^+$ (calcd for $C_{22}H_{18}N_3O_2$, 356.1399).

Cytotoxicity Assay. HCT116 and SW480 colon cancer cell lines were routinely maintained in Dulbecco's modified Eagle's medium, while the A375 melanoma cancer cell line was cultured in RPMI complete medium, both supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin. Culture reagents were provided by Lonza. Solutions of test compounds were prepared extemporaneously at 10^{-2} M by diluting the dried compound into the appropriate volume of DMSO. Tumor cells were counted using a CASY cell counter (Schärfe System) and seeded in 96-well plates at a density of 20×10^3 cells per well, allowing for exponential growth throughout the course of the experiment. After one night adhesion, cells were treated by addition of 100 μL of test compounds at different concentrations ranging from 10^{-10} to 10^{-4} M, obtained by serial dilution of a 10^{-2} M solution in supplemented culture medium. All concentrations were tested in triplicate. Cell viability was measured after 48 h treatment by addition of 20 μL of 1 mg/mL MTT. After 3 h of incubation, the medium was removed and formazan crystals diluted in 2-propanol/HCl, 39:1 v/v. Absorbance was measured using a microplate reader (Multiskan* EX, Thermo Scientific) at 540 nm. Data (absorbance as a function of log concentration) were fitted to a sigmoidal dose–response curve and analyzed using GraphPad Prism software. DMSO, MTT, 2-propanol, and HCl were from Sigma-Aldrich.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

This material (1D and 2D NMR, ESIMS, HRESIMS spectra of all reported new compounds) is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This contribution is part of the C2 component of the CRISP (Coral Reef Initiative in the South Pacific) project and granted by the Agence Française de Développement. We thank the Solomon Islands government for allowing us to collect in their country, and the Fisheries Department and R. Sulu (University of the South Pacific in Honiara) for their help and assistance. We thank the IRD diving team (SEOH) for the collection of the organisms. This study was also supported by the ECIMAR project ANR-06-BDIV-001-04. The authors wish to thank Dr. X. Turon (CEABCSIC, Spain) for identifying the ascidians. The spectroscopic experiments have been performed using the Biodiversité et Biotechnologies Marines (Bio2Mar, http://bio2mar.obs-banyuls.fr/fr/index.html) facilities at the University of Perpignan. C. Constant and Dr. G. Leguellinel are thanked for help with cell culture and IC_{50} determination, respectively. We are grateful to Prof. P. Cuq and Dr. J. Pannequin for providing access to cell culture facilities.

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