Bioactive Guanidine Alkaloids from Two Caribbean Marine Sponges

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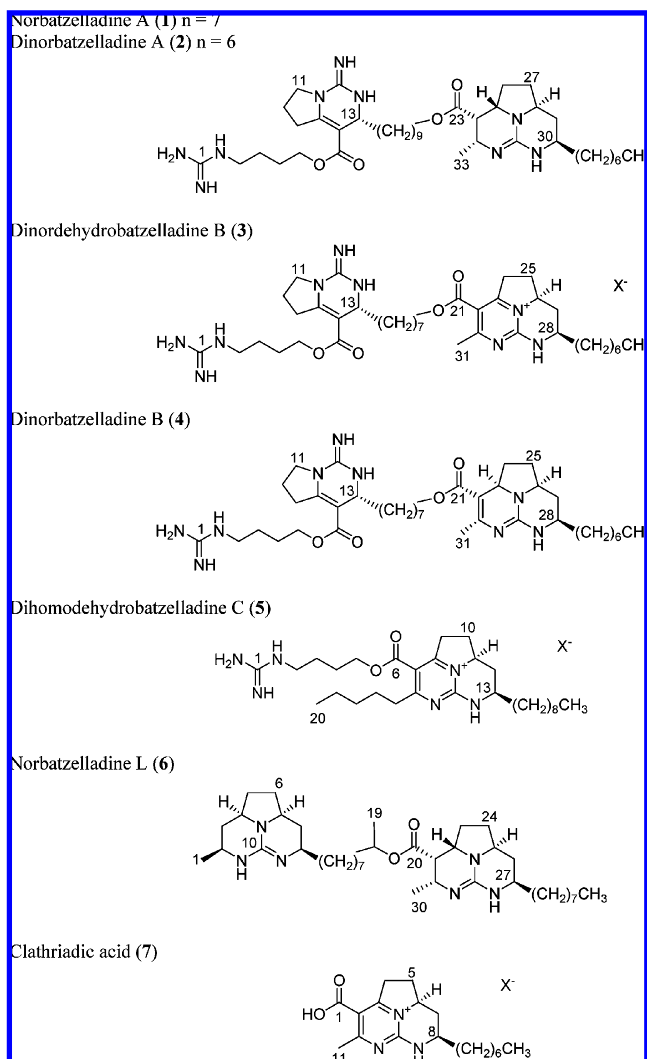
Received April 22, 2009

Seven new guanidine alkaloids (**1**−**7**) together with the known batzelladines A, F, H, and L, ptilomycalin A, and fromiamycalin were isolated from the Caribbean marine sponges *Monanchora arbuscula* and *Clathria calla*. Molecular structures were assigned on the basis of detailed analysis of 1D and 2D NMR spectra and mass spectrometry data, and bioactivities of the alkaloids were evaluated against human cancer cell lines and malaria protozoa.

Sessile marine sponges (Porifera) use different strategies to survive in the highly competitive marine environment, and chemical defense is a common approach. This chemical arsenal constitutes an important source of novel and biologically active secondary metabolites.[1] The discovery of ptilomycalin A, a unique pentacyclic guanidine alkaloid with antiviral and antifungal activities, generated considerable interest in assessing marine invertebrates, especially Poecilosclerida sponges, for new bioactive guanidine alkaloids.[2] A large number of polycyclic guanidine alkaloids have been reported so far, including the crambines; crambidine; crambescidins 359, 431, 657, 800, 816, 826, 830, 834, and 844; isocrambescidin 800; batzelladines A−N; celeromycalin; fromiamycalin; and ptilomycalin D.[3−8] Many of the polycyclic guanidine derivatives exhibit biological activities such as HIV gp120-human CD4-binding inhibition, p56^{lck}-CD4 dissociation induction, Ca^{2+} channel blocker activity, cytotoxicity, ichthyotoxicity, antifungal, antibacterial, and antimalarial activities.[6,8,9] The unique and fascinating structures of these metabolites, coupled with the wide-ranging bioactivities they usually display, have made them attractive targets for synthesis. Ptilomycalin A, crambescidins 359, 657, and 800, batzelladines A and D−F, and dehydrobatzelladine C have all been prepared through total syntheses, which in some cases prompted revisions of the original configurational assignments.[10−13] Additionally, biomimetic synthetic approaches have afforded clues regarding the biosynthesis of polycyclic guanidine alkaloids.[14]

As part of our search for bioactive marine natural products, we studied the chemical diversity of two specimens of the Caribbean marine sponge *Monanchora arbuscula* (de Laubenfels, 1953) collected in Martinique, using cytotoxicity assay-guided fractionation. Five new polycyclic guanidine alkaloids were obtained, norbatzelladine A (**1**), dinorbatzelladine A (**2**), dinordehydrobatzelladine B (**3**), dinorbatzelladine B (**4**), and dihomodehydrobatzelladine C (**5**) together with the known batzelladines A and L, ptilomycalin A, and fromiamycalin. The same process was applied to a specimen of the Caribbean marine sponge *Clathria calla* (de Laubenfels, 1934) collected in Guadeloupe, and the new norbatzelladine L (**6**) and clathriadic acid (**7**) together with the known ptilocaulin and batzelladines F, H, and L were identified. In this paper we describe the isolation, structure elucidation, and biological activity of the new compounds occurring naturally as the guanidinium salts. This study has also raised ecological and chemotaxonomical issues: the variation observed in the composition of the two *M. arbuscula* extracts and a further example of polycyclic guanidine alkaloids well known in *Monanchora* species, isolated from a *Clathria* sp. sponge.



Results and Discussion

Both *M. arbuscula* specimens, collected in November 2002 and April 2003 from the island of Martinique, were submitted to the same bioassay-guided fractionation procedure. $MeOH/CH_2Cl_2$ (1:1) extracts were subjected to diol-bonded phase flash chromatography, and bioactive fractions were further purified by C_{18} reversed-phase semipreparative HPLC. The first specimen of *M. arbuscula*

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10.1021/np900244g CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 09/10/2009

Table 1. ^{1}H NMR (500 MHz) Data for Compounds **1**−**4** in MeOH-d_4

no.	δ_H mult. (J in Hz)			
	1	**2**	**3**	**4**
2	3.23, t (6.9)	3.23, t (6.9)	3.23, t (6.9)	3.23, t (6.8)
3	1.69, m	1.69, m	1.68, m	1.69, m
4	1.77, m	1.77, m	1.76, m	1.77, m
5	4.22, t (6.3)	4.22, t (6.3)	4.22, t (6.5)	4.22, t (6.5)
9	3.32, m	3.32, m	3.32, m	3.32, m
	2.99, m	2.99, m	2.98, dt (18.0,9.5)	2.98, dt (18.1, 9.8)
10	2.23, m	2.23, m	2.24, m	2.23, m
	2.11, m	2.11, m	2.11, m	2.11, m
11	3.82, m	3.82, m	3.81, dt (9.2, 2.1)	3.82, dt (9.2, 2.1)
	3.67, dd (17.0, 10.1)	3.67, m	3.67, dt (17.0, 9.5)	3.68, dt (17.1, 9.5)
13	4.40, t (6.1)	4.40, t (6.1)	4.39, t (5.8)	4.40, t (6.3)
14	1.58, m	1.58, m	1.59, m	1.58, m
15	1.41, m	1.41, m	1.43, m	1.42, m
16	1.33, m	1.33, m	1.33, m	1.33, m
17	1.33, m	1.33, m	1.33, m	1.33, m
18	1.33, m	1.33, m	1.38, m	1.33, m
19	1.33, m	1.33, m	1.78, m	1.65, m
20	1.37, m	1.37, m	4.33, t (6.5)	4.14, m
21	1.65, m	1.65, m		
22	4.14, t (6.5)	4.14, t (6.5)		
23				4.53, dd (9.2, 6.4)
24	3.13, dd (4.7, 3.3)	3.13, dd (4.7, 3.3)	3.60, d (8.4)	2.53, ddd (12.0, 7.4, 4.1)
				1.67, m
25	3.95, m	3.95, m	2.65, dd (12.5, 6.4)	2.10, m
				1.57, m
26	2.24, m	2.24, m	4.57, m	3.82, m
	1.60, m	1.60, m		
27	2.22, m	2.22, m	2.58, dt (13.2, 3.5)	2.43, ddd (11.2, 6.7, 3.0)
	1.66, m	1.66, m	1.57, m	1.32, m
28	3.55, m	3.55, m	3.81, m	3.47, m
29	2.36, ddd (12.7, 4.9, 2.5)	2.36, ddd (12.7, 4.9, 2.5)		
	1.43, m	1.43, m		
30	3.54, m	3.54, m		
31			2.75, s	2.30, s
32	3.85, m	3.85, m	1.58, m	1.58, m
33	1.27, d (6.7)	1.27, d (6.7)	1.47, m	1.44, m
34	1.58, m	1.58, m	1.33, m	1.33, m
35	1.39, m	1.39, m	1.33, m	1.33, m
36	1.33, m	1.33, m	1.33, m	1.33, m
37	1.33, m	1.33, m	1.38, m	1.38, m
38	1.33, m	1.29, m	0.94, t (6.5)	0.90, t (7.0)
39	1.29, m	1.29, m		
40	1.29, m	0.90, t (7.0)		
41	0.90, t (7.0)			

yielded compounds **1**−**4** along with the known compounds batzelladine A and ptilomycalin A, while the second specimen provided the pure compounds **1**, **2**, and **5** along with the known compounds batzelladines A and L, ptilomycalin A, and fromiamycalin.

The sample of *C. calla* collected in July 2006 from the island of Guadeloupe was extracted with MeOH/CH_2Cl_2 (1:1) to provide a crude extract, which was subjected to C_{18} reversed-phase flash chromatography. The seven fractions obtained were screened for cytotoxic activity and submitted to HPLC-MS analyses. The bioactive MeOH/CH_2Cl_2 (1:1) fraction was then subjected to C_{18} reversed-phase semipreparative HPLC to yield the new compounds **6** and **7** together with the known ptilocaulin and batzelladines F, H, and L identified by comparison of the ESIMS and NMR spectra with literature data.[6]

Compound **1** was isolated as a colorless oil. A pseudomolecular ion in the positive HRESIMS at m/z 754.5705 $[M + H]^+$ allowed the molecular formula $C_{41}H_{71}N_9O_4$ to be assigned to **1**. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were very similar to those of batzelladine A.[6a] Both triplets at δ_H 4.22 (t, 6.3, H_2-5) and 4.14 (t, 6.5, H_2-22) were assigned to the oxygenated methylene of the ester functions C-5 and C-22, respectively. The nonequivalent protons at δ_H 3.82 (m, H-11a) and 3.67 (dd, 17.0, 10.1, H-11b) together with the triplet at δ_H 4.40 (t, 6.1, H-13) were characteristic of the left-hand bicycle of batzelladine A (C_7−C_{13} fragment). The methines at δ_H 3.13 (dd, 4.7, 3.3, H-24), 3.95 (m, H-25), 3.55 (m, H-28), 3.54 (m, H-30), and 3.85 (m, H-32) belonged to the tricyclic right-hand side of batzelladine A. According to the molecular formula, compound **1** corresponded to norbatzelladine A. Further HRESIMS/MS study allowed us to place the loss of the methylene unit. The fragmentation of the molecular ion peak at m/z 754.5705 afforded a fragment at m/z 336.2645 ($C_{19}H_{34}N_3O_2$ $[M - C_{22}H_{38}N_6O_2 + H]^+$ Δ 0.2 ppm) following a McLafferty rearrangement of the ester. Similar fragmentation patterns were observed for batzelladine A, and the presence of the corresponding fragment at m/z 350 confirmed the loss of the methylene unit at the right-hand side of **1**.[6,7] Compound **1** corresponded to the norbatzelladine A. The positions of the protons in the tricyclic core of **1** were deduced from the low values of the coupling constants for the ^{1}H NMR signals: $^{3}J_{H24-H25}$, $^{3}J_{H24-H32}$ (4.7, 3.3 Hz) and $^{3}J_{H29-H28}$, $^{3}J_{H29-H30}$ (4.9, 2.5 Hz) in accordance with the β position of H-32, H-24, and H-25 and with the α position of H-30, H-29a, and H-28, respectively. The *trans* relative stereochemistry of the tricyclic core was confirmed by the NOESY correlations H-28/H-27a, H-28/H-29a, and H-30/H-29a on one hand and H-24/H-32, H-24/H-25, H-25/H-26a, and H26a/H-27b on the other hand. Consequently, compound **1** showed the same relative configuration as batzelladine A.

Compound **2** was isolated as a colorless oil, and its molecular formula was determined as $C_{40}H_{70}N_9O_4$ (m/z 740.5547 $[M + H]^+$, Δ 0.2 ppm) by positive HRESIMS. The ^{1}H and ^{13}C NMR spectra

Table 2. ^{13}C NMR (125 MHz) Data for Compounds **1**−**3** in MeOH-d_4

	δ_C, mult.		
no.	**1**	**2**	**3**
1	158.6, qC	158.6, qC	158.6, qC
2	42.0, CH_2	42.0, CH_2	42.0, CH_2
3	26.6, CH_2	26.6, CH_2	26.6, CH_2
4	27.0, CH_2	27.0, CH_2	27.0, CH_2
5	65.2, CH_2	65.2, CH_2	65.2, CH_2
6	166.1, qC	166.1, qC	166.1, qC
7	103.3, qC	103.3, qC	103.3, qC
8	152.7, qC	152.7, qC	152.7, qC
9	32.0, CH_2	32.0, CH_2	32.0, CH_2
10	22.9, CH_2	22.9, CH_2	22.9, CH_2
11	48.8, CH_2	48.8, CH_2	48.8, CH_2
12	153.1, qC	153.1, qC	153.0, qC
13	51.3, CH	51.3, CH	51.3, CH
14	37.5, CH_2	37.5, CH_2	37.5, CH_2
15	25.3, CH_2	25.3, CH_2	25.3, CH_2
16	30.4, CH_2	30.4, CH_2	30.4, CH_2
17	30.4, CH_2	30.4, CH_2	30.4, CH_2
18	30.4, CH_2	30.4, CH_2	27.2, CH_2
19	30.4, CH_2	30.4, CH_2	29.7, CH_2
20	27.1, CH_2	27.1, CH_2	67.0, CH_2
21	29.8, CH_2	29.8, CH_2	164.7, qC
22	66.1, CH_2	66.1, CH_2	112.8, qC
23	170.7, qC	170.7, qC	166.9, qC
24	45.6, CH	45.6, CH	32.8, CH_2
25	57.8, CH	57.8, CH	30.1, CH_2
26	29.3, CH_2	29.3, CH_2	63.1, CH
27	31.4, CH_2	31.4, CH_2	31.0, CH_2
28	57.3, CH	57.3, CH	53.2, CH
29	34.2, CH_2	34.2, CH_2	152.2, qC
30	53.2, CH	53.2, CH	178.2, qC
31	151.5, qC	151.5, qC	26.4, CH_3
32	49.9, CH	49.9, CH	35.0, CH_2
33	18.4, CH_3	18.4, CH_3	25.6, CH_2
34	37.0, CH_2	37.0, CH_2	30.4, CH_2
35	26.2, CH_2	26.2, CH_2	30.4, CH_2
36	30.4, CH_2	30.4, CH_2	30.4, CH_2
37	30.4, CH_2	30.4, CH_2	23.5, CH_2
38	30.4, CH_2	33.0, CH_2	14.3, CH_3
39	33.0, CH_2	23.7, CH_2	
40	23.7, CH_2	14.4, CH_3	
41	14.4, CH_3		

(Tables 1 and 2) were similar to those of norbatzelladine A (**1**) and batzelladine A, and the molecular formula indicated that **2** was the lower homologue of **1**. HRESIMS/MS study showed a fragment at *m/z* 322.2478 ($C_{18}H_{32}N_3O_2$ $[M - C_{22}H_{38}N_6O_2 + H]^+$ Δ 3.3 ppm), consistent with a right-hand alkyl chain of seven carbons. On the basis of NOESY correlations and ^{1}H NMR coupling constants, the relative configuration of compound **2**, identified as dinorbatzelladine A, was the same as norbatzelladine A (**1**).

Compound **3** was isolated as a colorless oil, and its molecular formula was determined as $C_{38}H_{61}N_9O_4$ (*m/z* 708.4919 $[M + H]^+$ Δ −0.1 ppm) by positive HRESIMS. ^{1}H and ^{13}C NMR data (Tables 1 and 2) of the left-hand part of **3** were the same as those of **1** and **2**. Two missing methylene units were evidenced in accordance with the number of carbons in the molecular formula, and a characteristic ESIMS/MS fragment of the molecular ion at 274.2 $[M + H - C_{21}H_{36}N_6O_4]^+$, assigned to the decarboxylated tricyclic core of the molecule with a seven-carbon alkyl side chain as in batzelladine B, ascertained a seven-carbon central alkyl chain in **3**. Comparing with the right-hand tricyclic core for batzelladine A, the characteristic signals at δ_H 3.95 (m, H-25), 3.85 (m, H-32), and 1.27 (d, 6.7, H_3-33) were absent in the ^{1}H NMR spectrum of **3** and evidenced an additional unsaturation corresponding to an aromatization of the pyrimidinic part of the tricyclic core, in accordance with the ^{13}C NMR data modifications observed for the carbons at δ_C 112.8 (C-22), 166.9 (C-23), and 178.2 (C-30). Compound **3**, with two methylene units less than batzelladine B, was dinordehydrobatzelladine B, and it is the first example of an aromatic derivative of batzelladines A and B. According to ^{1}H NMR data, the relative configuration at C-13 was identical to those of **1** and **2**. In the right-hand tricycle, on the basis of the NOE correlation observed, the protons H-26 and H-28 were placed in a *cis* configuration.

Compound **4** was isolated as a colorless oil, and its molecular formula was determined as $C_{38}H_{63}N_9O_4$ (*m/z* 710.5074 $[M + H]^+$ Δ −0.2 ppm) by positive HRESIMS. According to the molecular formula, compound **4** had two methylene units less than batzelladine B, which had to occur in the central alkyl chain on the basis of the ESIMS/MS fragment of the molecular ion at *m/z* 274.2, as for compound **3**. The ^{1}H NMR data (Table 1) matched with the structure of batzelladine B. As in **3**, the characteristic signals of the usual tricyclic core in batzelladine A were absent in the ^{1}H NMR spectrum of **4**, and the new signals at δ_H 4.53 (dd, 9.2, 6.4, H-23) and 2.30 (s, H_3-31) evidenced a reduction in the tricyclic core, as in batzelladine B. NOESY experiment revealed the correlations between the protons at δ_H 3.82 (H-26), 2.10 (H-25a), 1.67 (H-24b), and 4.53 (H-23), therefore confirming the same relative configuration as batzelladine B. Compound **4** was identified as dinorbatzelladine B.

Compound **5** was isolated as a colorless oil, and its molecular formula of $C_{29}H_{50}N_6O_2$ was established by positive HRESIMS (*m/z* 515.4064 $[M + H]^+$ Δ −0.7 ppm). The ^{1}H and ^{13}C NMR spectra (Table 3) matched with those of the known dehydrobatzelladine C isolated from *M. unguifera*.[8b] The molecular formula indicated the presence of two extra methylene groups, but MS fragmentation did not allow determination of the lengths of the alkyl chains. The significant HMBC correlations H-16/C-15, H-16/C-17, and H-16/C-18 together with the chemical shifts of H_3-20 (δ_H 0.92, t, 7.1), H_2-18 (δ_H 1.39, m), and C-19 (δ_C 23.5) were consistent with the five-carbons alkyl chain at C-15 of dehydrobatzelladine C. Accordingly, the two additional methylenes were located on the second alkyl side chain, thus including nine carbons. The *cis* relative stereochemistry of the tricyclic core of **5** was assumed on the basis of the similarities with the chemical shifts of H-11 and H-13 reported in the literature for dehydrobatzelladine C and notably supported by the NOE correlations between H-11, H-13, H-12a, and H-12b. Compound **5** therefore corresponded to the dihomodehydrobatzelladine C.

Compound **6** was isolated as a colorless oil. The pseudomolecular ion in the positive HRESIMS/MS at *m/z* 639.5327 ($[M + H]^+$ Δ 3.0 ppm), after the trifluoroacetic acid (TFA) counterion dissociation (*m/z* 753.5257 for $C_{40}H_{68}N_6O_4F_3$ $[M + H + TFA]^+$ Δ 1.1 ppm), allowed the molecular formula $C_{38}H_{66}N_6O_2$ to be assigned to **6**. The ^{1}H and ^{13}C NMR data matched those of the known batzelladine L,[7,12] and the molecular formula indicated the loss of one methylene unit. HRESIMS[3] fragmentation of the molecular ion $[M + H]^+$ allowed the distribution of the methylenes between the two alkyl chains. The characteristic fragment observed at *m/z* 336.2656 ($C_{19}H_{34}N_3O_2^+$ Δ 3.0 ppm) was in accordance with an eight-carbon alkyl side chain and a nine-carbon central alkyl chain. The *cis* relative stereochemistry of the right-hand tricyclic core of **6** was suggested on the basis of the similarities with the chemical shifts of H-11 and H-13 reported for dehydrobatzelladines F, G, and L,[6b,8b] and especially supported by the NOE correlations: H-21/H-22, H-21/H-29, H-22/H-29, and H-22/H-23a indicated the *cis* relative position of H-21, H-22, and H-29, and H-25/H-23b and H-25/H-24a indicated the *trans* relative position of H-22 and H-25. The H-2/H-4, H-4/H-9, and H-7/H-9 NOE correlations indicated the *cis* relative stereochemistry position of the protons H-2, H-4, H-7, and H-9 of the left-hand tricyclic core. So, compound **6** corresponded to the norbatzelladine L, also isolated in small amount from the specimen of *M. arbuscula* 2003.

Compound **7** was isolated as a colorless oil. The pseudomolecular ion in the positive HRESIMS/MS at *m/z* 318.2173 (M^+ Δ −0.9 ppm) allowed the molecular formula $C_{18}H_{28}N_3O_2$ to be assigned to

Table 3. NMR (500 MHz) Data for Compounds **5**−**7** in MeOH-d_4

	dihomodehydrobatzelladine C (**5**)		norbatzelladine L (**6**)		clathriadic acid C (**7**)	
no.	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	162.8, qC		20.7, CH_3	1.28, d (6.5)	163.1, qC	
2	42.0, CH_2	3.24, t (7.5)	47.3, CH	3.55, m	112.5, qC	
3	26.9, CH_2	1.70, m	36.9, CH_2	2.25, m	166.9, qC	
				1.26, m		
4	26.6, CH_2	1.84, m	57.6, CH	3.75, m	32.9, CH_2	3.66, dd (19.7, 8.9)
5	66.5, CH_2	4.40, t (6.6)	31.1, CH_2	2.23, m	30.3, CH_2	2.65, m
				1.69, m		
6	164.7, qC		31.0, CH_2	2.21, m	62.7, CH	4.57, m
				1.69, m		
7	112.7, qC		57.5, CH	3.75, m	31.0, CH_2	2.57, dt (13.5, 3.7)
						1.57, m
8	166.9, qC		34.8, CH_2	2.27, m	53.0, CH	3.81, ddd (11.7, 8.3, 4.0)
				1.24, m		
9	34.3, CH_2	3.60, dd (18.1, 8.6)	51.6, CH	3.43, m	151.9, qC	
		3.42, dd (10.9, 8.4)				
10	30.3, CH_2	2.66, m	151.1, qC		178.2, qC	
		2.00, m				
11	63.1, CH	4.60, m	35.9, CH_2	1.57, m	26.0, CH_3	2.76, s
12	31.0, CH_2	2.58, dt (13.2, 3.5)	26.2, CH_2	1.34, m	35.3, CH_2	1.58, m
		1.58, m				
13	53.3, CH	3.80, m	30.5, CH_2	1.34, m	25.6, CH_2	1.47, m
14	152.3, qC		30.5, CH_2	1.34, m	30.6, CH_2	1.33, m
15	181.3, qC		30.5, CH_2	1.34, m	30.6, CH_2	1.33, m
16	38.6, CH_2	3.00, t (7.7)	26.6, CH_2	1.35, m	30.6, CH_2	1.33, m
17	29.1, CH_2	1.71, m	36.7, CH_2	1.63, m	23.7, CH_2	1.38, m
				1.56, m		
18	32.7, CH_2	1.39, m	73.4, CH	4.98, m	14.4, CH_3	0.91, t (7.0)
19	23.5, CH_2	1.37, m	20.4, CH_3	1.26, d (6.2)		
20	14.2, CH_3	0.92, t (7.1)	170.3, qC			
21	35.1, CH_2	1.85, m	45.6, CH	3.10 (dd 4.5, 3.5)		
		1.67, m				
22	26.0, CH_2	1.47, m	58.0, CH	3.95, m		
23	30.6, CH_2	1.30, m	29.2, CH_2	2.26, m		
				1.65, m		
24	30.6, CH_2	1.30, m	31.4, CH_2	2.20, m		
				1.64, m		
25	30.6, CH_2	1.30, m	57.3, CH	3.55, m		
26	30.4, CH_2	1.30, m	34.2, CH_2	2.36, ddd (12.5, 4.7, 2.0)		
				1.45, m		
27	33.1, CH_2	1.30, m	53.2, CH	3.54, m		
28	23.7, CH_2	1.31, m	151.5, qC			
29	14.4, CH_3	0.90, t (6.9)	49.9, CH	3.85, m		
30			18.5, CH_3	1.26, d (6.2)		
31			37.0, CH_2	1.63, m		
				1.56, m		
32			26.2, CH_2	1.34, m		
33			30.5, CH_2	1.34, m		
34			30.5, CH_2	1.34, m		
35			30.5, CH_2	1.34, m		
36			33.0, CH_2	1.34, m		
37			23.7, CH_2	1.38, m		
38			14.4, CH_3	0.90, t (7.1)		

7. The ^{1}H and ^{13}C NMR data matched those of several metabolites of *M. arbuscula*. The chemical shifts at δ_H 4.57 (m, H-6), 3.81 (ddd, 11.7, 8.3, 4.0, H-8), 3.66 (dd, 19.7, 8.9, H-4), 2.76 (s, H-11), 2.65 (m, H_2-5), 2.57 (dt, 13.5, 3.7, H-7a), and 1.57 (m, H-7b) were characteristic of an aromatic tricyclic guanidinium salt. The ESIMS/MS fragment at *m/z* 274.2 was assigned to the decarboxylated tricyclic core with a seven-carbon alkyl side chain. ^{1}H and ^{13}C NMR chemical shifts in the aromatic tricyclic guanidinium in clathriadic acid (**7**) were nearly identical to those in dinordehydrobatzelladine B (**3**), suggesting the same stereochemistry of the aromatic tricyclic guanidinium, in accordance with the NOESY correlation observed between H-6 and H-8.

The chemical compositions of the two specimens of *M. arbuscula* collected from the same place in 2002 and 2003 were different. The batzelladine A derivatives (bicyclic left-hand and tricyclic right-hand cores) were present in both specimens, while the batzelladine F derivatives (tricyclic right- and left-hand cores) were present only in the specimen collected in April 2003. The reasons of these changes are not clear, and the factors influencing the production of the secondary metabolites remain unknown: season, reproduction cycle, environmental stress, etc. A great variability in morphology and spicule complement has been reported in this species.[15a] The involvement of symbiotic microorganisms in the biosynthesis of these metabolites or some of their precursors should also be considered.

This study on *M. arbuscula* supports the suggestion proposed in 2000 to group the sponges of the genera *Monanchora*, *Batzella*, and *Crambe* in one sponge genus, preferentially *Crambe*, because of the similarity of their morphological characters and secondary metabolites.[7] Nevertheless, in the last updated Porifera classification,[15b] the order Poecilosclerida was divided into four suborders: Latrunculina, Microcionina, Myxillina, and Mycalina. The suborder Myxillina includes *Monanchora* and *Crambe* (Crambeidae), *Batzella* (Chondropsidae), and *Phorbas* (Hymedesmiidae). All these sponges classified in different families are known to produce polycyclic guanidine alkaloids, and the tricyclic guanidine alkaloids could be considered as taxonomic chemomarkers of the

Table 4. Cytotoxicity of Compounds **1**−**3** and **5**−**7** (μM) against Cancer Cell Lines

compound	breast (MDA-MB-231)			lung NSCLC (A549)			colon (HT29)		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
norbatzelladine A (**1**)	3.8	6.4	11.4	2.1	4.6	8.6	1.6	3.2	5.7
dinorbatzelladine A (**2**)	3.0	3.8	4.6	4.9	5.1	5.4	1.9	4.2	7.6
dinordehydrobatzelladine B (**3**)	n.d.[a]	n.d.	n.d.	7.9	>14	>14	6.2	>14	>14
dihomodehydrobatzelladine C (**5**)	6.1	9.8	15.6	4.7	8.2	13.1	3.1	5.1	8.2
norbatzelladine L (**6**)	0.7	1.9	4.8	1.1	2.1	4.2	1.2	2.2	4.0
clathriadic acid (**7**)	13.5	>30	>30	>30	>30	>30	>30	>30	>30

[a] n.d.: not determined.

Table 5. *In Vitro* Antimalarial Activity of Compounds **1**−**3**, **5**−**7**, Ptilomycalin A, and Batzelladines A and L (μM) against *Plasmodium falciparum* FcBl

compound	*P. falciparum* (FcB1) IC_{50}	HeLa cells TC_{50}	selective index TC_{50}/IC_{50}
norbatzelladine A (**1**)	0.2	4.7	23.5
dinorbatzelladine A (**2**)	0.9		
dinordehydrobatzelladine B (**3**)	0.8		
dihomodehydrobatzelladine C (**5**)	4.5		
norbatzelladine L (**6**)	0.4		
clathriadic acid (**7**)	2.3		
batzelladine A	0.3	2.9	9.7
batzelladine L	0.3	<0.1	<1
ptilomycalin A	0.1	0.1	1.4

suborder Myxillina. However, these markers do not appear entirely specific. Moreover, one species in the suborder Mycalina (*Arenochalina mirabilis*, Mycalidae) is reported to produce mirabilins A−F (ptilocaulin derivatives),[17] and mirabilin G was isolated from an Australian *Clathria* sp.[16] Our results on *C. calla* also indicate the presence of these alkaloids in a representative of the suborder Microcionida.

The compounds reported here were isolated through their cytotoxic activity, and as the antimalarial activity of related compounds has been reported,[8b,9c] the compounds were tested for both activities. Preliminary structure−activity relationships in this class of compounds may be deduced from the antitumor and antimalarial bioassays. Compound **4** was not assayed because of the small amount of pure compound available. In the antitumor assay (Table 4), in accordance with previous results in anti-HIV screenings,[6] the presence of a single polycyclic guanidine moiety prevented display of high activity. Clathriadic acid (**7**), with one tricyclic guanidine core, was the least active ($GI_{50} > 10$ μM), but with the guanidine ester (**5**) the cytotoxic activity increased (GI_{50} 3−6 μM). Compounds **1**, **2**, and **3** with bicyclic and tricyclic guanidine cores showed mild antitumor activity (GI_{50} 3−7 μM). Compared to **1** and **2**, the aromatization of the tricyclic guanidine core in compound **3** decreased the activity against the three tumor cell lines. The most active compound was **6**, with two tricyclic guanidine cores, notably against the breast cancer cell line MDA-MB-231 ($GI_{50} = 0.7$ μM).

The aromatization in the tricyclic core of **3** (compared to **1**, **2**) and **5** (compared to batzelladine L) did not alter the antimalarial activity (Table 5). Batzelladine A, with one bicyclic and one tricyclic guanidine core, tested for the first time against malaria (FcB1 strain), was as active as norbatzelladine A (**1**), batzelladine L, and norbatzelladine L (**6**), the last two both having two tricyclic guanidine cores (IC_{50} 0.2 to 0.4 μM, FcB1). Ptilomycalin A, with one pentacyclic guanidine core, was the most active (IC_{50} 0.1 μM), while compound **5** and **7**, with one aromatic tricyclic guanidine core, exhibited weak antimalarial activities, IC_{50} 2.3 and 4.5 μM, respectively. Batzelladine L and ptilomycalin A exhibited similar antimalarial activities against the FcB1 strain than against the D6 and W2 strains.[8b] The selectivity index (SI) was used as the parameter of clinical significance of the test samples by comparing general toxins and the selective inhibitory effect on *P. falciparum*[18] calculated here as TC_{50} (HeLa)/IC_{50} (*P. falciparum*). Ptilomycalin A showed high toxicity against HeLa cells ($TC_{50} < 0.08$ μM) in accordance with reported data for the D6 strain, while the noncytotoxicity against Vero cells induced the high SI value reported.[8a] The low SI of ptilomycalin A was indicative of a too toxic principle, while the highest SI value of compound **1** made it more suitable for *in vivo* screening as a potential antiprotozoa lead. Batzelladine L showed a comparable IC_{50} with ptilomycalin A but with a higher SI value.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter using a 10 cm microcell (1 mL). UV measurements were performed on a Varian Cary 300 Scan UV−visible spectrometer. IR spectra were obtained with a Perkin-Elmer Paragon 1000 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with 500 MHz Bruker Avance NMR spectrometers. Chemical shifts (δ) are recorded in ppm with CD_3OD (δ_H 3.31 ppm and δ_C 49.0 ppm) as internal standards with multiplicity (s singlet, d doublet, t triplet, m multiplet). Positive or negative electrospray ionizations (±ESI) were performed on a Bruker Esquire 3000 Plus mass spectrometer. High-resolution mass spectra were obtained from an Orbitrap spectrometer (ThermoFinnigan). HPLC purifications were carried out on a Waters equipment (pump 600 E, autoinjector 417, and photodiode array detector 996) coupled with an evaporative light-scattering detector (ELSD) SEDEX 55.

Sponge Material. The marine sponge *Monanchora arbuscula* (Duchassaing & Michelotti, 1864) (Crambeidae, Myxillina, Poecilosclerida), senior synonym of *Monanchora unguifera* (de Laubenfels, 1953), was collected at 18 m depth by scuba off the island of Martinique from the Rocher du Diamant, in July 2002 (first specimen) and in April 2003 (second specimen). The marine sponge *Clathria calla* (de Laubenfels, 1934) (Microcionidae, Microcionina, Poecilosclerida) was collected at 25 m depth by scuba off the island of Guadeloupe from the Pointe de Gros Morne, in July 2006. Each specimen was immediately frozen after harvesting and kept at −18 °C until extraction. Voucher samples ORMA 8522, ORMA 25522 (*M. arbuscula*), and ORMA 044069 (*C. calla*) were deposited at Pharmamar (Spain).

Extraction and Isolation. *M. arbuscula* First Specimen. The wet sponge (130 g) was extracted with CH_2Cl_2/MeOH (1:1), and the crude oil (3.8 g) was partitioned between H_2O and CH_2Cl_2. The bioactive organic layer (287 mg) was then subjected to diol-bonded phase flash chromatography successively eluted by CH_2Cl_2, CH_2Cl_2/MeOH (8:2), and MeOH. Both last fractions showed bioactivity and similar HPLC-MS profiles. The CH_2Cl_2/MeOH fraction (105 mg) was purified by semipreparative reversed-phase HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 μm, eluting from MeOH/H_2O/TFA, 75:25:0.1 to 90:10:0.1) to afford pure compounds **1** (1.5 mg), **2** (2.1 mg), **3** (0.8 mg), and **4** (0.9 mg) along with batzelladine A (2.6 mg) and ptilomycalin A (4.6 mg).

***M. arbuscula* Second Specimen.** The wet sponge (89 g) was extracted with CH_2Cl_2/MeOH (1:1), and the crude oil (3.5 g) was partitioned between H_2O and CH_2Cl_2. The bioactive organic layer (771 mg) was then subjected to diol-bonded silica gel flash chromatography successively eluted with CH_2Cl_2, CH_2Cl_2/MeOH (8:2), and MeOH. The CH_2Cl_2/MeOH fraction (467 mg) was also purified by semipreparative reversed-phase HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 μm, eluting with MeOH/H_2O/TFA, 50:50:0.1 to 80:20:0.1, to afford pure compounds **1** (2.4 mg), **3** (1.6 mg), and batzelladine A (5 mg) along with a mixture of ptilomycalin A and compound **5** (1 mg), subsequently purified by semipreparative reversed-phase HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 μm, eluting with CH_3CN/H_2O/TFA (68:32:0.1

to 75:25:0.1). Compounds **2** and **4** were identified in the CH_2Cl_2/MeOH fraction by HPLC/MS analysis.

***C. calla* Specimen.** The wet sponge (98 g) was extracted with CH_2Cl_2/MeOH (1:1) and the crude oil (5 g) subjected to C_{18}-bonded phase flash chromatography using a decreasing polarity step gradient from MeOH/H_2O, 0:1 to 1:0, to CH_2Cl_2/MeOH, 0:1 to 1:0. The seven fractions obtained were screened for cytotoxicity and submitted to HPLC-MS analyses. The bioactive fraction CH_2Cl_2/MeOH, 1:1, was subjected to C_{18} reversed-phase semipreparative HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 μm, MeOH/H_2O/TFA, 60:40:0.1 to 75:25: 0.1) to yield a mixture of fromiamycalin and compound **6**, compound **7** (2.5 mg), ptilocaulin (4.4 mg), and batzelladines F (10.6 mg), H (5 mg), and L (1.3 mg). Compound **6** was finally purified (2.7 mg) by semipreparative HPLC (Phenomenex Luna phenyl-hexyl, 250 × 10 mm, 5 μm, CH_3CN/H_2O/TFA, 45:55:0.1 to 70:30:0.1)

Norbatzelladine A (1): colorless oil; $[\alpha]^{24}_D$ +5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.52), 253 (3.80) nm; IR (film) ν_{max} 3600−3000, 3000−2800, 1726, 1679, 1640, 1460, 1423, 1199, 1176, 1126, 829, 798, 718 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; (+) HRESIMS *m/z* 754.5705 (calcd for $C_{41}H_{72}N_9O_4$, 754.5712).

Dinorbatzelladine A (2): colorless oil; UV (MeOH) λ_{max} (log ε) 292 (4.19), 254 (3.65) nm; IR (film) ν_{max} 3600−3000, 3000−2800, 1692, 1679, 1454, 1431, 1199, 1178, 1132, 835, 798, 718 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; (+) HRESIMS *m/z* 740.5547 (calcd for $C_{40}H_{70}N_9O_4$, 740.5556).

Dinordehydrobatzelladine B (3): colorless oil; $[\alpha]^{24}_D$ +28 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 291 (3.21), 258 (3.56) nm; IR (film) ν_{max} 3600−3000, 3000−2800, 1682, 1635, 1553, 1452, 1276, 1250, 1196, 1173, 1126, 835, 798, 718 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; (+) HRESIMS *m/z* 708.4919 (calcd for $C_{38}H_{62}N_9O_4$, 708.4929).

Dinorbatzelladine B (4): colorless oil; $[\alpha]^{24}_D$ +10 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 289 (3.31), 257 (3.44) nm; IR (film) ν_{max} 3600−3000, 3000−2800, 1679, 1648, 1553, 1269, 1258, 1199 1178, 1129, 832, 798, 718, 667 cm^{-1}; ^1H NMR see Table 1; an accident resulting in loss of the compound prevented accumulation of ^{13}C NMR data; (+) HRESIMS *m/z* 710.5074 (calcd for $C_{38}H_{64}N_9O_4$, 710.5086).

Dihomodehydrobatzelladine C (5): colorless oil; $[\alpha]^{24}_D$ +19 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 306 (2.86), 259 (3.69) nm; IR (film) ν_{max} 3600−3000, 3000−2800, 1677, 1633, 1553, 1452, 1258, 1199, 1173, 1129, 829, 798, 716 cm^{-1}; ^1H and ^{13}C NMR see Table 3; (+) HRESIMS *m/z* 515.4064 (calcd for $C_{29}H_{51}N_6O_2$, 515.4077).

Norbatzelladine L (6): colorless oil; $[\alpha]^{24}_D$ −2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 295 (3.52), 257 (3.88) nm; IR (film) ν_{max} 3450, 3000, 1675, 1633, 1553, 1460, 1258, 1190, 1173, 1129, 798, 725 cm^{-1}; ^1H and ^{13}C NMR see Table 3; (+) HRESIMS *m/z* 639.5327 (calcd for $C_{38}H_{67}N_6O_2$, 639.5330).

Clathriadic Acid (7): colorless oil; $[\alpha]^{24}_D$ +13 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 306 (3.14), 242 (3.61) nm; IR (film) ν_{max} 3450, 3000, 1680, 1623, 1573, 1454, 1258, 1187, 1163, 1129, 829, 783, 723 cm^{-1}; ^1H and ^{13}C NMR see Table 3; (+) HRESIMS *m/z* 318.2173 (calcd for $C_{18}H_{28}N_3O_2$, 318.2183).

Antitumor Assay. A colorimetric assay using sulforhodamine B was adapted for a quantitative measurement of cell growth and viability following a technique described in the literature.[19] *In vitro* cytotoxicity was evaluated against three tumor cell lines: lung carcinoma A549, colon carcinoma HT29, and breast MDA-MB-231.

Antimalarial Assay. The antimalarial activity was evaluated against *Plasmodium falciparum* FcB1 (chloroquine-resistant strain from Colombia) according to a reported methodology.[20]

Acknowledgment. We are grateful to PharmaMar Madrid for antitumor screening and for financial support also provided by a grant (R.L.) of the Région Provence-Alpes-Côte d'Azur. We wish to thank S. Prado for antimalarial screening (MNHN, Paris), J. M. Guigonis and M. Gaysinski for HRMS^n and NMR experiments, respectively (Université de Nice), and Mr le Préfet de la Martinique, Mr le Préfet de la Guadeloupe, and DIREN for assistance in collecting the marine sponge.

Supporting Information Available: NMR and HRESIMS data for compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900244G