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Unguiculin A and Ptilomycalins E–H, Antimalarial Guanidine Alkaloids from the Marine Sponge *Monanchora unguiculata*

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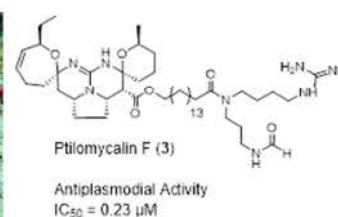
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Supporting Information

ABSTRACT: Chemical study of the CH₂Cl₂–MeOH (1:1) extract from the sponge *Monanchora unguiculata* collected in Madagascar highlighted five new compounds, one acyclic guanidine alkaloid, unguiculin A (1) and four pentacyclic alkaloids, ptilomycalins E–H (2–5), along with four known compounds: crambescidin 800 (6) and crambescidin 359 (7), crambescidic acid (8), and fromiamycalin (9). Their structures were elucidated by 1D and 2D NMR spectra and HRESIMS data. All compounds were evaluated for their cytotoxicity against KB cells and their antiplasmodial activity. The new ptilomycalin E (2) and the mixture of the new ptilomycalins G (4) and H (5) showed promising cytotoxicity against KB cells with IC₅₀ values of 0.85 and 0.92 μM, respectively. Ptilomycalin F (3) and fromiamycalin (9) exhibited promising activity against *Plasmodium falciparum* with IC₅₀ values of 0.23 and 0.24 μM, respectively.

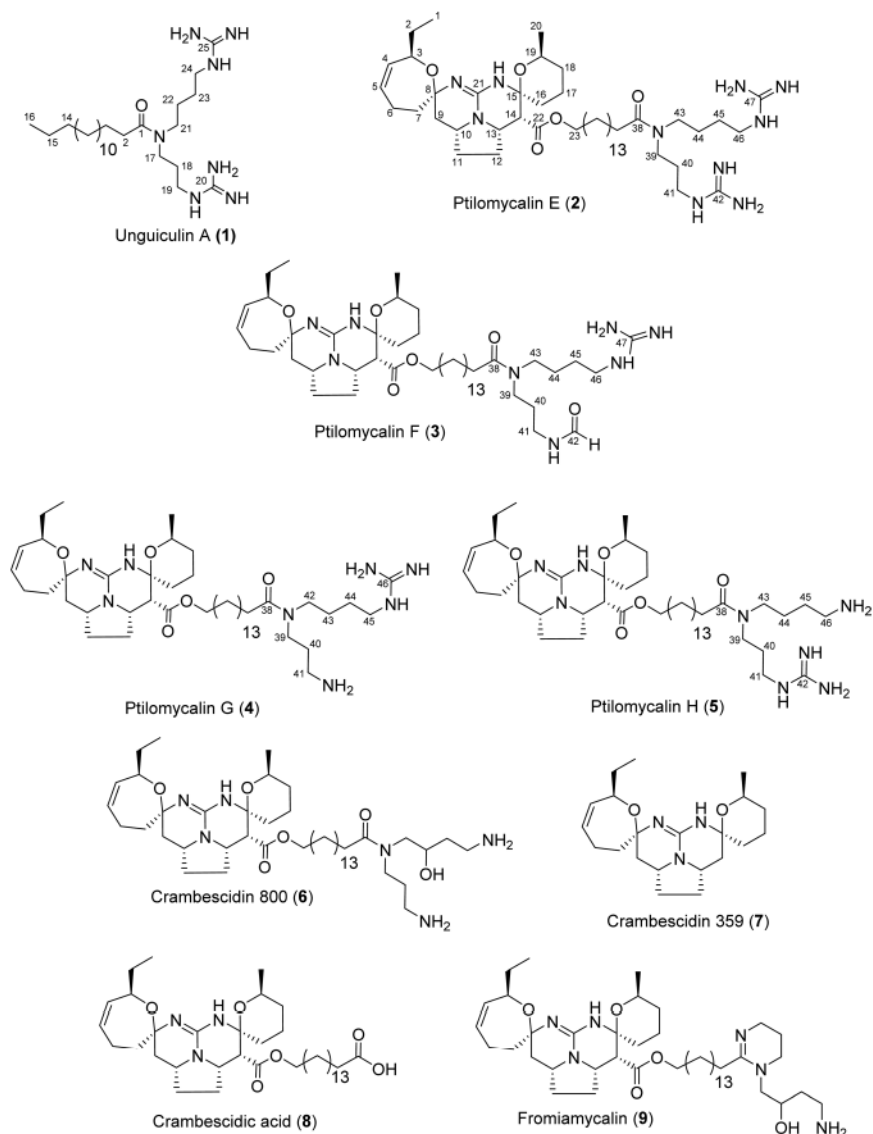


Ptilomycalins, crambescidins, monanchomycalins, monanchocidins, normonanchocidins, neofolitispates, fromiamycalin, and celeromycalin are the common names of a class of alkaloids with a pentacyclic skeleton usually linked by a linear ω-hydroxy fatty acid to a spermidine or hydroxyspermidine unit. At least 30 pentacyclic compounds have been reported from sponges, seven in the order Poecilosclerida: *Monanchora dianchora* (also known as *Neofolitispia dianchora*),^{1,2} *Monanchora pulchra*,^{3–7} *Monanchora* sp.,^{8,9} *Monanchora unguiculata*,^{10,11} *Monanchora arbuscula* (also known as *Monanchora unguifera*),^{12–14} *Crambe crambe*,¹⁵ and *Hemimycala* sp.¹⁶ Another one belongs to the order Axinellida: *Ptilocaulis* aff. *spiculifer*.¹⁶ However, a new examination of a voucher specimen of *Ptilocaulis* aff. *spiculifer* (Harbor Branch collection) has led to a re-evaluation of the taxonomy and therefore this sponge fits to the Poecilosclerid genus *Batzella* Topsent, 1891. Some of these compounds have also been reported from starfishes of the order Valvatida, namely, *Fromia monilis* and *Celerina heffernani*.¹⁷ Many of these compounds were mentioned as possessing noteworthy biological activities including cytotoxicity, and antibacterial, antifungal, antimalarial, and antiprotozoal activities.^{12,18–20}

In our continuing search for bioactive metabolites from marine invertebrates,^{21–23} the sponge *Monanchora unguiculata* (Dendy, 1922) from the order Poecilosclerida was investigated. The extract was found to have cytotoxic activity against KB cells and antiplasmodial activity against *Plasmodium falciparum*. Previous studies on *M. unguiculata* collected first on the south side of St. Joseph's Atoll, Amirantes Islands (Republic of the Seychelles) in 1992–93 by Braeckman et al.¹⁰ and then at Male Atoll in the Maldivian Islands in 2004 by Meragelman et al.¹¹ led to the isolation of crambescidins 359 and 431 for the first study and crambescidin acid and monanchorin for the second one. No relevant biological activity has been reported on these molecules.

Chemical investigation of the extract of a new batch of *M. unguiculata* collected in Mitsio islands, Madagascar, led to the isolation of the known compounds crambescidin 800 (6), crambescidin 359 (7), crambescidic acid (8), fromiamycalin (9), together with a new acyclic guanidine alkaloid (1) and four new pentacyclic alkaloids (2–5). The isolation, structure elucidation, and biological characterization of these new compounds are described herein. All compounds were isolated as the formate ion salts.

Chart 1



RESULTS AND DISCUSSION

The CH_2Cl_2 -MeOH extract of the freeze-dried sponge was found to be highly cytotoxic against the KB tumor cell line (99.0% inhibition at $10 \mu\text{M}$ concentration) and to exhibit promising *in vitro* antiplasmodial activity ($\text{IC}_{50} < 5 \mu\text{g}/\text{mL}$). The red gum active extract was subjected to a combination of normal-phase MPLC and repetitive reversed-phase preparative and semipreparative HPLC to yield the salts of nine compounds (1–9). The isolated secondary metabolites were identified as crambescidin 800 (6),²⁴ crambescidin 359 (7),¹⁰ crambescidic acid (8),¹⁴ and fromiamycalin (9)¹⁷ by comparison with published spectroscopic data. Besides the known compounds isolated, the procedure afforded one acyclic guanidine alkaloid, unguiculin A (1) and four pentacyclic alkaloids, ptilomycalins E–H (2–5) described below.

Unguiculin A (1) was obtained as a yellow oil. Its molecular formula was established by HRESIMS to be $\text{C}_{25}\text{H}_{53}\text{N}_7\text{O}$. Analysis of the 1D and 2D ^1H and ^{13}C NMR data for 1

(CD_3OD , Table 1) revealed resonances and correlations (Figure 1) consistent with those of a fatty acid chain with one terminal methyl (δ_{C} 14.2, δ_{H} 0.90) and a carbonyl group (δ_{C} 175.6). HMBC correlations between C-1 (δ_{C} 175.6) and H-2 (δ_{H} 2.38), H-17 (1a: δ_{H} 3.41; 1b: δ_{H} 3.42), and H-21 (δ_{H} 3.37) clearly indicated that the carbonyl group is attached to a spermidine moiety and a hexadecanoyl chain. As was described by Ohtani et al.¹⁶ for the ptilomycalin A, the spermidine moiety exists in two rotamers (1a–1b). Rotational isomers are indeed characteristic of tertiary amides. However, the two rotamers could not be fully defined by NOEs due to signal overlap in the ^1H NMR spectrum. The propyl chain of the spermidine was determined by COSY correlations from H-18 (1a: δ_{H} 1.79; 1b: δ_{H} 1.89) to H-17 (1a: δ_{H} 3.41; 1b: δ_{H} 3.42) and H-19 (1a: δ_{H} 3.15; 1b: δ_{H} 3.21). The butyl chain of the spermidine was determined by COSY correlations from H-21 (1a,b: δ_{H} 3.37) to H-22 (1a,b: δ_{H} 1.64) and from H-23 (1a,b: δ_{H} 1.57) to H-24 (1a,b: δ_{H} 3.21); by HMBC correlations between H-23 and C-22; but also by TOCSY correlations between H-21 to H-22, H-23, and H-24. HMBC correlation between H-19 (1a: δ_{C} 39.8, δ_{H} 3.15; 1b: δ_{C} 39.5, δ_{H} 3.21) and C-20 (1a,b: δ_{C} 158.4) indicated that the ending methylene of the propyl chain was attached to a guanidine

Table 1. 1D and 2D NMR Spectroscopic Data (^1H 500 MHz, ^{13}C 125 MHz, CD_3OD) for Unguiculin A (1a–b)

position	δ_{C} , type	δ_{H} (J in Hz)	COSY ($^1\text{H}-^1\text{H}$)	HMBC ($^1\text{H}-^{13}\text{C}$)
1	175.6, C	-	-	-
2	33.7, CH_2	2.38, t (7.1)	3	1, 3, 4
3	26.4, CH_2	1.61, m	2, 4	1, 2, 4
4–13	30–31, CH_2	1.30, m	-	-
14	33.0, CH_2	1.30, m	-	15, 16
15	23.5, CH_2	1.31, m	16	14, 16
16	14.2, CH_3	0.90, t (6.9)	15	14, 15
17	43.8, CH_2 (1a)	3.41, m (1a)	18	1, 18, 19, 21
	46.0, CH_2 (1b)	3.42, m (1b)		
18	28.0, CH_2 (1a)	1.79, quint (6.8) (1a)	17, 19	17, 19
	28.9, CH_2 (1b)	1.89, quint (7.0) (1b)		
19	39.8, CH_2 (1a)	3.15, t (6.9) (1a)	18	17, 18, 20
	39.5, CH_2 (1b)	3.21, t (6.8) (1b)		
20	158.4, C	-	-	-
21	48.5, CH_2 (1a)	3.37, m (1a)	22	1, 22
	45.8, CH_2 (1b)	3.37, m (1b)		
22	26.9, CH_2	1.64, m	21	21
23	26.8, CH_2	1.57, m	24	22
24	41.9, CH_2	3.21, t (6.8)	23	22, 23, 25
25	158.4, C	-	-	-

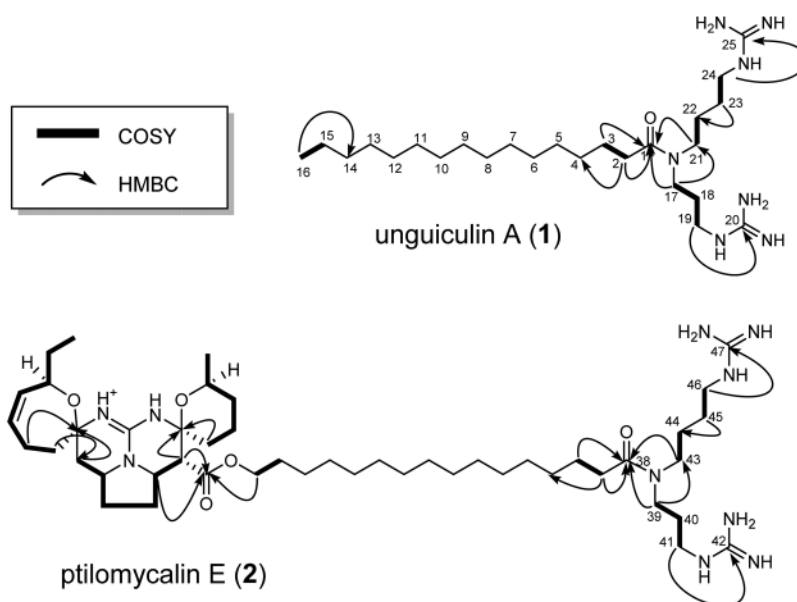


Figure 1. Key COSY and HMBC correlations for compounds 1 and 2.

moiety and the correlation between H-24 (1a,b: δ_{C} 41.9, δ_{H} 3.21) and C-25 (1a,b: δ_{C} 158.4) indicated that the ending methylene of the butyl chain was attached to another guanidine moiety. The length of the linear fatty chain (14 methylenes) was deduced thanks to the molecular formula.

Ptilomycalin E (2) was obtained as a yellow oil. Its molecular formula was established by HRESIMS to be $\text{C}_{47}\text{H}_{84}\text{N}_{10}\text{O}_5$. Analysis of the NMR data for 2 (Table 2) revealed resonances and correlations (Figure 1) consistent with those of a pentacyclic guanidine-like structure linked to a fatty chain by an ester bond as in crambescidin 800 (6)^{10,24} and fromiamycalin (9):¹⁷ two methyl groups C-1 and C-20 (δ_{H} 0.87, 1.10; δ_{C} 10.9, 21.9), two olefinic carbons C-4 and C-5 (δ_{H} 5.52, 5.72; δ_{C} 134.4, 131.2), two oxymethines C-3 and C-19 (δ_{H} 4.45, 3.87; δ_{C} 72.2, 68.3), two N-substituted CH groups C-10 and C-13 (δ_{H} 4.06, 4.36; δ_{C} 55.4, 53.9), one oxymethylene C-23 (δ_{H} 4.14; δ_{C} 66.5), two

nonprotonated carbons of aminocarbinols C-8 and C-15 (δ_{C} 82.1, 85.1), a guanidine-like carbon C-21 (δ_{C} 150.4), and one ester carbonyl group (δ_{C} 170.1). Further COSY and HMBC correlations completed the arguments for the pentacyclic part. Analysis of the COSY revealed the presence of the spins systems C-1–C-2–C-3–C-4–C-5–C-6–C-7, C-9–C-10–C-11–C-12–C-13–C-14, and C-16–C-17–C-18–C-19–C-20. HMBC correlations between H-6 and C-8, H-7 and C-9, and H-9 and C-8 indicated the position of the nonprotonated carbon C-8. HMBC correlations between H-13, H-14, H-23, and C-22 indicated the position of the ester carbonyl group C-22. HMBC correlations between H-14, H-16, and C-15 indicated the position of the nonprotonated carbon C-15. The relative configuration of the pentacyclic guanidine part was assumed to be the same that of the crambescidins as indicated by the close similarities of the chemical shifts and coupling constants. On the

Table 2. ^1H and ^{13}C NMR Spectroscopic Data (^1H 500 MHz, ^{13}C 125 MHz, CD_3OD) for Ptilomycalins E–F (2–3)

position	Ptilomycalin E (2a–2b)		Ptilomycalin F (3a–3b)	
	δC , type	δH (J in Hz)	δC , type	δH (J in Hz)
1	10.9, CH_3	0.87, t (7.3)	10.8, CH_3	0.85, t (7.3)
2	29.6, CH_2	1.47, m	30.3, CH_2	1.47, m
	-	-	-	1.56, m
3	72.2, CH	4.45, br d (9.4)	72.3, CH	4.45, br d (9.5)
4	134.4, CH	5.52, br d (11.0)	134.3, CH	5.52, br d (11.0)
5	131.2, CH	5.72, br dd (9.9, 7.8)	131.3, CH	5.72, m
6	24.5, CH_2	2.17, m	24.5, CH_2	2.16, m
	-	2.46, m	-	2.44, m
7	37.9, CH_2	2.00, m	38.2, CH_2	2.00, m
	-	2.49, m	-	2.36, m
8	85.1, C	-	85.1, C	-
9	37.8, CH_2	1.43, m	37.8, CH_2	1.42, m
	-	2.65, dd (13.0, 4.8)	-	2.65, dd (12.9, 4.8)
10	55.4, CH	4.06, m	55.6, CH	4.07, m
11	31.5, CH_2	1.61, m	31.4, CH_2	1.61, m
	-	2.31, m	-	2.31, m
12	27.6, CH_2	1.83, m	27.5, CH_2	1.82, m
	-	2.33, m	-	2.35, m
13	53.9, CH	4.36, dt (9.2, 5.4)	54.2, CH	4.36, dt (9.5, 5.6)
14	51.2, CH	3.06, d (5.1)	50.7, CH	3.08, d (5.0)
15	82.1, C	-	82.2, C	-
16	32.8, CH_2	1.72, m	32.9, CH_2	1.7, m
17	19.4, CH_2	1.78, m	19.4, CH_2	1.77, m
	-	2.00, m	-	1.82, m
18	33, CH_2	1.28, m	32.9, CH_2	1.29, m
	-	1.72, m	-	1.7, m
19	68.3, CH	3.87, m	68.4, CH	3.83, m
20	21.9, CH_3	1.10, d (6.2)	21.8, CH_3	1.10, d (6.2)
21	150.4, C	-	150.3, C	-
22	170.1, C	-	170.2, C	-
23	66.5, CH_2	4.14, m	66.5, CH_2	4.14, m
24	27, CH_2	1.66, m	29.6, CH_2	1.66, m
25	27.1, CH_2	1.38, m	27.1, CH_2	1.36, m
26–35	30–31, CH_2	1.31, m	30–31, CH_2	1.31, m
36	26.7, CH_2	1.63, m	26.8, CH_2	1.60, m
37	33.9, CH_2	2.40, m	34.0, CH_2	2.37, m
38	175.7, C	-	175.7, C	-
39	44.2, CH_2 (2a)	3.42, m (2a)	46.9, CH_2	3.36, m
	46.1, CH_2 (2b)	3.43, m (2b)	-	-
40	28.2, CH_2 (2a)	1.82, m (2a)	30.0, CH_2	1.82, m
	29.2, CH_2 (2b)	1.90, m (2b)	-	-
41	40.1, CH_2 (2a)	3.18, t (6.7) (2a)	36.5, CH_2 (3a)	3.26, t (6.8) (3a)
	39.8, CH_2 (2b)	3.23, m (2b)	36.6, CH_2 (3b)	3.21, t (6.8) (3b)
42	158.7, C	-	163.9, CH (3a)	8.08, s (3a)
	-	-	164.0, CH (3b)	7.98, s (3b)
43	49.0, CH_2 (2a)	3.4, m	46.2, CH_2	3.36, m
	46.3, CH_2 (2b)	-	-	-
44	27.1, CH_2	1.66, m	25.8, CH_2	1.60, m
45	27.1, CH_2	1.61, m	27.1, CH_2	1.62, m
46	42.1, CH_2	3.23, m	42.1, CH_2	3.21, m
47	158.7, C	-	158.7, C	-

basis of the literature, the chemical shift of H-14 α for 2 (δ_{H} 3.06, d, $J = 5.1$ Hz) is characteristic of a *syn* relationship between H-10 and H-13, while in isocrambescidin 800, H-14 presented a deshielded chemical shift (δ_{H} 3.80, d, $J = 3.4$ Hz).⁹ The following key NOE correlations are also in agreement with this configuration: from H-13 to H-10 and H-14. Overall, an excellent correlation between the NMR data of 2 and 1 evidenced that the fatty acid chain includes one terminal carbonyl group (δ_{C} 175.7) linked to a spermidine moiety. Like for unguiculin A (1) the spermidine moiety of 2 exists in two rotamers (2a–2b). Analysis of the COSY, the TOCSY and the HMBC revealed the presence of the propyl spin system C-39–C-40–C-41 and the butyl spin system C-43–C-44–C-45–C-46 (see Table 2 for δ_{H} and δ_{C} values). HMBC correlations also indicated that the ending methylene of the propyl chain (C-41) and the butyl chain (C-46) were both attached to a guanidine moiety for C-42 and C-47. The number of methylenes of the fatty chain was determined by the molecular formula (15 CH_2), as for crambescidin 800 (6),^{10,24} fromiamycalin (9),¹⁷ crambescidic acid (8),¹⁴ and ptilomycalin A.¹⁶

Ptilomycalin F (3) was obtained as a yellow oil. Its molecular formula was established by HRESIMS to be $\text{C}_{47}\text{H}_{82}\text{N}_8\text{O}_6$. A comparison of the ^1H and ^{13}C NMR data with those of ptilomycalin E (2) indicated that the guanidine moiety attached to the propyl group of 2 is replaced by a formamide group in 3. The HMBC experiment clearly showed a correlation between the methylene H-41 (3a: δ_{H} 3.26; 3b: δ_{H} 3.21) and C-42 (3a: δ_{C} 164.0; 3b: δ_{C} 163.9) as well as between the formamide H-42 (3a: δ_{H} 8.08; 3b: δ_{H} 7.98) and C-41 (3a: δ_{C} 36.5; 3a: δ_{C} 36.6).

Ptilomycalins G and H (4–5) were structural isomers and were obtained in a mixture as a yellow oil. RP-18-UPLC-HRMS analysis revealed approximately a 2:1 mixture. Their common molecular formula ($\text{C}_{46}\text{H}_{82}\text{N}_8\text{O}_5$) was established by HRESIMS. A comparison of the ^1H and ^{13}C NMR (^{13}C NMR chemical shifts were determined from the HSQC and HMBC spectra) data with those of ptilomycalin E (2) and ptilomycalin F (3) supported a pentacyclic guanidine-like structure with a fatty acid chain. Comparison of the spectroscopic data of the N-terminal fatty acid moiety of ptilomycalin G (4) and ptilomycalin E (2) revealed good similarities for the butyl group. However, the chemical shifts for the propyl group, especially for H-41 (4a: δ_{H} 2.98; 4b: δ_{H} 2.88; 2a: δ_{H} 3.18; 2b: δ_{H} 3.23) were different. Moreover, in contrast to compound 2, no correlation between H-41 and a nonprotonated carbon was observed on the HMBC spectrum. These spectroscopic features as well as the molecular formula established by HRESIMS ($\text{C}_{46}\text{H}_{82}\text{N}_8\text{O}_5$) supported that the propyl group of the amide moiety was not modified with a terminal guanidine moiety like compound 2, but simply by a primary amine group.

The same comparison was made between ptilomycalin II (5) and ptilomycalin E (2). So differences were observed for the chemical shifts of the butyl group especially for H-46 (5: δ_{H} 2.96; 2: δ_{H} 3.23). Moreover, no correlation was observed between H-46 and a nonprotonated carbon. The butyl group of the N-terminal fatty acid moiety of 5 was thus terminated by a primary amine.

The *in vitro* activities of unguiculin A (1), ptilomycalins E (2), F (3), the mixture of ptilomycalins G and H (4–5), crambescidin 800 (6), and fromiamycalin (9) were evaluated against KB cells and against *Plasmodium falciparum* (Table 4). Several isolated compounds exhibited strong cytotoxicity, particularly ptilomycalin E (2), the mixture of ptilomycalins G and H (4, 5) and fromiamycalin (9) with IC_{50} of 0.85, 0.92, and 1.17 μM ,

Table 3. ^1H and ^{13}C NMR Spectroscopic Data (^1H 500 MHz, ^{13}C 125 MHz, CD_3OD) for Ptilomycalins G–H (4–5)^a

position	Ptilomycalin G (4a–4b)		Ptilomycalin H (5a–5b)	
	δC , type	δH (J in Hz)	δC , type	δH (J in Hz)
1	10.7, CH ₃	0.85, t (7.3)	10.7, CH ₃	0.85, t (7.3)
2	30.1, CH ₂	1.46, m	30.1, CH ₂	1.46, m
	-	1.55, m	-	1.55, m
3	72.1, CH	4.41, br d (10.3)	72.1, CH	4.41, br d (10.3)
4	134.1, CH	5.51, br d (10.9)	134.1, CH	5.51, br d (10.9)
5	131.1, CH	5.71, m	131.1, CH	5.71, m
6	24.3, CH ₂	2.16, m	24.3, CH ₂	2.16, m
	-	2.42, m	-	2.42, m
7	38.0, CH ₂	1.98, m	38.0, CH ₂	1.98, m
	-	2.38, m	-	2.38, m
8	84.9, C	-	84.9, C	-
9	37.7, CH ₂	1.42, m	37.7, CH ₂	1.42, m
	-	2.64, dd (12.8, 4.7)	-	2.64, dd (12.8, 4.7)
10	55.4, CH	4.06, m	55.4, CH	4.06, m
11	31.3, CH ₂	1.60, m	31.3, CH ₂	1.60, m
	-	2.30, m	-	2.30, m
12	27.4, CH ₂	1.81, m	27.4, CH ₂	1.81, m
	-	2.32, m	-	2.32, m
13	54.0, CH	4.35, dt (9.7, 5.3)	54.0, CH	4.35, dt (9.7, 5.3)
14	50.7, CH	3.07, d (5.0)	50.7, CH	3.07, d (5.0)
15	81.9, C	-	81.9, C	-
16	32.7, CH ₂	1.7, m	32.7, CH ₂	1.7, m
17	19.2, CH ₂	1.76, m	19.2, CH ₂	1.76, m
	-	1.82, m	-	1.82, m
18	32.8, CH ₂	1.28, m	32.8, CH ₂	1.28, m
	-	1.7, m	-	1.7, m
19	68.2, CH	3.83, m	68.2, CH	3.83, m
20	21.7, CH ₃	1.09, d (6.1)	21.7, CH ₃	1.09, d (6.1)
21	150.3, C	-	150.3, C	-
22	170.0, C	-	170.0, C	-
23	66.4, CH ₂	4.14, m	66.4, CH ₂	4.14, m
24	29.4, CH ₂	1.65, m	29.4, CH ₂	1.65, m
25	26.9, CH ₂	1.36, m	26.9, CH ₂	1.36, m
26–35	30–31, CH ₂	1.31, m	30–31, CH ₂	1.31, m
36	26.6, CH ₂	1.60, m	26.6, CH ₂	1.60, m
37	33.7, CH ₂	2.39, m	33.7, CH ₂	2.39, m
38	176.3, C	-	176.3, C	-
39	46.1, CH ₂ (4a)	3.42, m (4a)	49.1, CH ₂ (5a)	3.40, m
	43.0, CH ₂ (4b)	3.46, t (6.5) (4b)	43.9, CH ₂ (5b)	
40	27.9, CH ₂ (4a)	1.95, m (4a)	29.0, CH ₂ (5a)	1.89, m (5a)
	26.6, CH ₂ (4b)	1.90, m (4b)	28.0, CH ₂ (5b)	1.80, m (5b)
41	38.0, CH ₂ (4a)	2.98, m (4a)	39.6, CH ₂ (5a)	3.22, m (5a)
	37.9, CH ₂ (4b)	2.88, t (6.9) (4b)	39.9, CH ₂ (5b)	3.15, t (6.8) (5b)
42	48.5, CH ₂	3.38, m	158.5, C	-
43	26.6, CH ₂	1.68, m	48.5	3.38, m
44	26.8, CH ₂	1.60, m	25.7, CH ₂	1.67, m
45	42.0, CH ₂	3.22, m	26.7, CH ₂	1.63, m
46	158.5, C	-	40.2, CH ₂	2.96, m

^a ^{13}C NMR chemical shifts were determined from the HSQC and HMBC spectra.

respectively. As for the antimalarial activity, it seems to be correlated to the pentacyclic moiety; unguiculin A, the acyclic compound showed lower activity (IC_{50} 12.89 μM) than

Table 4. Biological Activities in Vitro (KB Cell Line Cytotoxicity and Antiplasmodial Activity) for Pure Isolated Natural Products

compound	KB cell line	<i>P. falciparum</i> (3D7)	selectivity index
	IC_{50} , μM	IC_{50} , μM	
Unguiculin A (1)	7.66 (± 0.96)	12.89 (± 0.07)	0.6
Ptilomycalin E (2)	0.85 (± 0.03)	0.35 (± 0.13)	2.4
Ptilomycalin F (3)	1.61 (± 0.19)	0.23 (± 0.01)	7.0
Ptilomycalins G + H (4 + 5)	0.92 (± 0.05)	0.46 (± 0.05)	2.0
Crambescidin 359 (6)	-	-	-
Crambescidin 800 (7)	1.31 (± 0.07)	0.52 (± 0.02)	2.5
Crambescidic acid (8)	8.80 (± 1.47)	-	-
Fromiamycalin (9)	1.17 (± 0.11)	0.24 (± 0.03)	4.9
Artemisinin	-	0.004 (± 0.001)	-

pentacyclic compounds. Crambescidin 800 (6), fromiamycalin (9), ptilomycalin E (2), ptilomycalin F (3), and the mixture of ptilomycalins G and H (4, 5) exhibited IC_{50} of 0.52, 0.24, 0.35, 0.23, and 0.46 μM , respectively. Lazaro et al.²⁰ suggested that the pentacyclic moiety was cytotoxic, whereas the spermidine-containing aliphatic chain increased antimalarial activity. Herein the results suggested that both pentacyclic moiety and the spermidine-containing aliphatic chain were involved in the cytotoxicity against KB cells and the antimalarial activity, but modifications in the spermidine-containing aliphatic chain allowed modulations of the activities. The spermidine-containing aliphatic chain of ptilomycalin E (2) seems to be more effective for a cytotoxic activity against KB cells and the spermidine-containing aliphatic chain of ptilomycalin F seems to be more effective for an antimalarial activity. The selectivity index (SI) was used as the parameter of clinical significance of the test samples by comparing the cytotoxicity on the KB cell line and the selective inhibitory effect on *P. falciparum* calculated here as $\text{IC}_{50}(\text{KB})/\text{IC}_{50}(\text{P. falciparum})$. As requested by the Medicine for Malaria Venture,²⁴ several of these compounds present an IC_{50} below the μM range. Ptilomycalin F (3) and fromiamycalin (9) showed similar antimalarial activity, but ptilomycalin F (3) showed the highest SI value (7.0), close to the value requested by the Medicine for Malaria Venture (SI > 10). KB cells are usually more sensitive than normal cells, so this value makes it more suitable for *in vivo* screening as a potential antiprotozoan lead.

EXPERIMENTAL SECTION

General Experiment Procedures. Optical rotations were measured on a MCP 300 Anton Paar modular circular polarimeter at 25 °C. ^1H and ^{13}C NMR data were acquired with a Bruker UltraShield Avance-500 or a Varian Inova 500 MHz spectrometers. Chemical shifts were referenced using the corresponding solvent signals (δ_{H} 3.31 and δ_{C} 49.00 for CD_3OD). The spectra were processed using 1D and 2D MestReNova (Mnova 10.0 Mestrelab Research) software. HRESIMS spectra were recorded using a Waters Acquity BEH C18, 1.7 μm , 150 \times 2.1 mm column on a Thermo Scientific Exactive Plus Orbitrap mass spectrometer or a Waters Micromass LCT-Premier TOF mass spectrometer with a Waters Acquity UPLC system.

MPLC separations were carried out on Buchi Sepacore flash systems C-605/C-615/C-660 and glass columns (460 \times 26 mm i.d.; 460 \times 36 mm i.d. and 230 \times 15 mm i.d.) packed with Macherey-Nagel MN Kieselgel silica gel (60–200 μm). Precoated TLC sheets of silica gel 60, Alugram SIL G/UV254 were used, and spots were visualized on the basis of the UV absorbance at 254 nm and by heating silica gel plates sprayed with formaldehyde–sulfuric acid or Dragendorff reagents. Analytical HPLC was carried out using a Gemini C₁₈ (150 \times 4.6 mm i.d., 3 μm) column, a Waters Sunfire C₁₈ (150 \times 4.6 mm i.d., 5 μm) column, or a Waters Xbridge C₁₈ (250 \times 4.6 mm i.d., 5 μm) column and was

performed on an Agilent 1100 series system controller equipped with a photodiode array detector (Agilent 1100 G1315B) and a mass spectrometer detector (Agilent 1100 G1956A) with Chemstation software. Preparative HPLC was carried out using a Waters Sunfire Prep RP₁₈ (150 × 19 mm i.d., 5 μm) column and was performed on a Waters 600 system controller equipped with a photodiode array detector (Waters 2996). Semipreparative HPLC was carried out using Waters Sunfire Prep RP₁₈ (250 × 10 mm i.d., 5 μm), Waters XBridge C₁₈ Prep (250 × 10 mm i.d., 5 μm), or Phenomenex Gemini C₁₈ prep (250 × 10 mm i.d., 5 μm) columns and was performed on a Waters 600 system controller equipped with photodiode array detectors (Waters 2996 and Waters 486). All solvents were analytical or HPLC grade and were used without further purification.

Animal Material. The sponge *M. unguiculata* (phylum Porifera, class Demospongiae, order Poecilosclerida, family Crambeidae) was collected in December 2012 in Mitsio Islands, Madagascar (13°29,032' S, 47°58,721' E at 2–6 m depth). One voucher specimen (RMNH POR 8722) was deposited in the Naturalis, Netherlands Centre for Biodiversity. Sponge samples were frozen immediately and kept at –20 °C until processed.

Extraction and Isolation. The frozen sponge (73 g) was chopped into small pieces and extracted exhaustively by maceration with CH₂Cl₂–MeOH (1:1 v:v) (2 × 1.5 L, each 24 h) at room temperature. After evaporating the solvents under reduced pressure, a red, oily residue (25.8 g) was obtained. The extract (25.8 g) was then subjected to MPLC over silica gel in a glass column (460 × 36 mm i.d.), eluting with a combination of isohexane, EtOAc, and MeOH of increasing polarity (20 mL min^{–1}). Five fractions were obtained: F1 eluted with isohexane–EtOAc (95:5) over 30 min; F2 eluted with isohexane–EtOAc (50:50) over 30 min; F3 eluted with EtOAc over 30 min; F4 eluted with EtOAc–MeOH (50:50) over 30 min; and F5 eluted with MeOH over 30 min.

Separation of fraction F3 (1.91 g) by MPLC on silica gel in a glass column (460 × 26 mm i.d.), using 20 mL min^{–1} gradient elution with 5% EtOAc–isohexane to 50% EtOAc–isohexane over 20 min; 50% EtOAc–isohexane over 10 min; 50% EtOAc–isohexane to 100% EtOAc over 20 min; 100% EtOAc over 10 min; 0% MeOH–EtOAc to 50% MeOH–EtOAc over 20 min; 50% MeOH–EtOAc over 10 min; 50% MeOH–EtOAc to 100% MeOH over 20 min; 100% MeOH over 10 min gave seven fractions (F31 to F37). F36 (619 mg) was also separated by MPLC over silica gel in a glass column (230 × 15 mm i.d.), using a combination of EtOAc and MeOH as eluents and gave four fractions (F361 to F364). The fraction F363 (45.1 mg) was subjected to semipreparative HPLC (Sunfire C₁₈ Prep Column, 5 μm, 250 × 10 mm i.d., 4.5 mL min^{–1} gradient elution with 2% CH₃CN–H₂O (+0.1% formic acid) to 35% CH₃CN–H₂O (+0.1% formic acid) over 20 min; UV 254 nm) to furnish pure compounds 1 (unguiculin A, 3.5 mg) and 2 (ptilomycalin E, 14 mg).

Fraction F4 (3.16 g) was subjected to preparative HPLC (Waters Sunfire Prep C₁₈ Column, 5 μm, 150 × 19 mm i.d., 18 mL min^{–1} gradient elution with 20% CH₃CN–H₂O (+0.1% formic acid) to 35% CH₃CN–H₂O (+0.1% formic acid) over 15 min, then 35% CH₃CN–H₂O (+0.1% formic acid) over 10 min and 35% CH₃CN–H₂O (+0.1% formic acid) to 60% CH₃CN–H₂O (+0.1% formic acid) over 5 min; UV 205 nm) and six fractions (F41 to F46) was collected. F42 was subjected to a subsequent semipreparative HPLC separation (4.5 mL min^{–1} isocratic elution with 29% CH₃CN–H₂O (+0.1% formic acid) over 20 min; UV 205 nm) to furnish four fractions (F421 to F424). F423 was subjected to a subsequent semipreparative HPLC separation (4.5 mL min^{–1} isocratic elution with 35% CH₃CN–H₂O (+0.1% TFA) over 20 min; UV 205 nm) to give pure compound 7 (crambescidin 359, 5 mg). F43 (245 mg) was subjected to semipreparative HPLC (Waters XBridge C₁₈ Prep Column, 5 μm, 250 × 10 mm i.d., 4.7 mL min^{–1} gradient elution with 30% CH₃CN–H₂O (+0.1% formic acid) over 19 min, then 30% CH₃CN–H₂O (+0.1% formic acid) to 50% CH₃CN–H₂O (+0.1% formic acid) over 2 min and 50% CH₃CN–H₂O (+0.1% formic acid) over 5 min; UV 205 nm) and seven fractions (F431 to F437) were collected. F436 afforded pure compound 2 (ptilomycalin E, 13 mg). F432 (39 mg) was subjected to a subsequent semipreparative HPLC separation (Waters XBridge C₁₈ Prep Column, 5 μm, 250 × 10 mm i.d., 4.7 mL min^{–1} gradient elution with 48% CH₃CN–H₂O (+0.1% TFA) to

56% CH₃CN–H₂O (+0.1% TFA) over 30 min; UV 205 nm) to furnish five fractions (F432a to F432e). F432d was a mixture of 4 and 5 (ptilomycalin G and H, 6 mg) and F432e contained the pure compound 2 (ptilomycalin E, 9 mg). F437 (10 mg) was subjected to a subsequent semipreparative HPLC separation (Waters XBridge C₁₈ Prep Column, 5 μm, 250 × 10 mm i.d., 4.7 mL min^{–1} gradient elution with 52% CH₃CN–H₂O (+0.1% TFA) to 64% CH₃CN–H₂O (+0.1% TFA) over 20 min; UV 205 nm) to give pure compound 3 (ptilomycalin F, 2.7 mg). Fraction F46 (42 mg) was subjected to a subsequent semipreparative HPLC separation (Phenomenex Gemini C₁₈ 5 μm, 250 × 10 mm i.d. column, 4.5 mL min^{–1} gradient elution 50% CH₃CN–H₂O (+0.1% formic acid) to 95% CH₃CN–H₂O (+0.1% formic acid) over 30 min; UV 205 nm) and led to the isolation of pure compound 8 (crambescidin acid, 1.1 mg).

Fraction F5 (1.51 g) was subjected to preparative HPLC (Waters Sunfire Prep C₁₈ Column, 5 μm, 150 × 19 mm i.d., 18 mL min^{–1} gradient elution with 20% CH₃CN–H₂O (+0.1% formic acid) to 35% CH₃CN–H₂O (+0.1% formic acid) over 15 min, then 35% CH₃CN–H₂O (+0.1% formic acid) over 10 min and 35% CH₃CN–H₂O (+0.1% formic acid) to 60% CH₃CN–H₂O (+0.1% formic acid) over 5 min; UV 205 nm) and six fractions (F51 to F56) were collected. The fractions F53 and F54 (79 mg) were subjected together to semipreparative HPLC (Waters XBridge C₁₈ Prep Column, 5 μm, 250 × 10 mm i.d., 4.7 mL min^{–1} gradient elution with 45% CH₃CN–H₂O (+0.1% TFA) to 70% CH₃CN–H₂O (+0.1% TFA) over 40 min; UV 205 nm) to furnish pure compounds 6 (crambescidin 800, 12 mg) and 9 (fromiamycalin, 2.6 mg).

Unguiculin A (1). Yellow oil; ¹H and ¹³C NMR data, Table 1; HRESIMS *m/z* 468.43820 [M + H]⁺ (calcd for C₂₅H₅₄N₇O, 468.43844).

Ptilomycalin E (2). Yellow oil; [α]_D²⁰ –3.8 (*c* 6.9, MeOH); IR (film): ν_{max} 2924, 1626, 1579, 1342, 1015 cm^{–1}; ¹H and ¹³C NMR data, Table 2; HRESIMS *m/z* 869.67041 [M + H]⁺ (calcd for C₄₇H₈₅N₁₀O₅, 869.66989).

Ptilomycalin F (3). Yellow oil; [α]_D²⁰ –5.0 (*c* 2.2, MeOH); ¹H and ¹³C NMR data, Table 2; HRESIMS *m/z* 428.32483 [M + 2H]²⁺ (calcd for C₄₇H₈₄N₈O₆, 428.32514).

Ptilomycalins G (4) and H (5) (approximately 2:1 mixture). Yellow oil; [α]_D²⁰ –3.2 (*c* 5.3, MeOH); ¹H and ¹³C NMR data, Table 3; HRESIMS *m/z* 827.64819 [M + H]⁺ (calcd for C₄₆H₈₃N₈O₅, 827.64809).

In Vitro Cytotoxicity Assay against the KB Cell Line. Cell proliferation was measured with CellTiter 96 Aqueous One solution reagent (Promega), and results are expressed as the percentage of inhibition of cellular proliferation of KB cells treated for 72 h with compounds compared to cells treated with DMSO only (mean ± SE of triplicate). The IC₅₀ determinations were performed in duplicate experiments and are expressed as individual values. Details of the protocol have been previously reported.⁹

In Vitro Antiplasmodial Assays. The *P. falciparum* strains utilized and details of the assay protocols have been previously reported.^{25,26} The selectivity index was determined as the ratio of cytotoxicity over antiplasmodial activity (expressed in IC₅₀ values).

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b01079.

Copies of HRMS–HRMS spectra, NMR spectra (¹H, ¹³C), and additional data (tables of NMR data) (PDF)

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Notes

The authors declare no competing financial interest.

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on April 3, 2017, with Chart 1 missing. The corrected version was reposted on April 10, 2017.