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

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Pierre-Eric Campos,† Jean-Luc Wolfender,§ Emerson F. Queiroz,§ Laurence Marcourt,§ Ali Al-Mourabit,‖ Michel Frederich,‖ Annélise Bordignon,‡ Nicole De Voogd,‖ Bertrand Illien,† and Anne Gauvin-Bialecki*,†

†Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Faculté des Sciences et Technologies, Université de La Réunion, 15 Avenue René Cassin, CS 92003, 97744 Saint-Denis Cedex 9, La Réunion, France
‡Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Sud, Université Paris-Saclay, 1, av. de la Terrasse, 91198 Gif-sur-Yvette, France
§School of Pharmaceutical Sciences, EPFL, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland
‖Laboratory of Pharmacognosy, Department of Pharmacy, CIRM, University of Liège B36, 4000 Liège, Belgium
§Naturalis Biodiversity Center, Darwinweg 2, 2333 CR Leiden, Netherlands

**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 fromiamycin (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 fromiamycin (9) exhibited promising activity against *Plasmodium falciparum* with IC₅₀ values of 0.23 and 0.24 μM, respectively.

Ptilomycalins, crambescidins, monanchomycalins, monanchocidins, etronanchocidins, neooffitipates, fromiamycins, and celeromycalins are the common names of a class of alkaloids with a pentacyclic skeleton usually linked by a linear ortho-hydroxy fatty acid to a spermidine or hydroxy spermidine unit. At least 30 pentacyclic compounds have been reported from sponges, seven in the order Poecilosclerida: *Monanchora dianchora* (also known as Neofolitisa dianchora),¹,² Monanchora pulchra,³–⁵ Monanchora sp.,⁶–⁹ Monanchora unguiculata,¹⁰,¹¹ Monanchora arbuscula (also known as Monanchora unguifera),¹²–¹⁴ Crambe crambae,¹⁵ and Hemimyctus sp.¹⁶ Another one belongs to the order Axinellida: *Ptilocaulis aff. spiciferus*.¹⁶ However, a new examination of a voucher specimen of *Ptilocaulis aff. spiciferus* (Harbor Branch collection) has led to a re-evaluation of the taxonomy and therefore this sponge fits to the Poecilosclerid genus *Batella* Topsent, 1891. Some of these compounds have also been reported from starfishes of the order Valvatida, namely, *Fornia monilis* and *Celerina heffernani*.¹⁷ Many of these compounds were mentioned as possessing noteworthy biological activities including cytotoxicity, and antibacterial, antifungal, antimalarial, and antiparasitic activities.¹⁵,¹⁶–²⁰

In our continuing search for bioactive metabolites from marine invertebrates,²¹–²⁵ the sponge *Monanchora unguiculata* (Dendy, 1922) from the order Poecilosclerida was investigated. The extract was found to have cytotoxic activity against KB cells and antimalarial 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 Maldives 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), fromiamycin (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.
RESULTS AND DISCUSSION

The CH₂Cl₂–MeOH extract of the freeze-dried sponge was found to be highly cytotoxic against the KB tumor cell line (99.0% inhibition at 10 μM concentration) and to exhibit promising in vitro antiplasmodial activity (IC₅₀ < 5 μg/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 crambesicidin 800 (6), crambesicidin 359 (7), crambesicid acid (8), and fromiamycalin (9) 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, ptilomycalin E–H (2–5) described below.

Unguiculin A (1) was obtained as a yellow oil. Its molecular formula was established by HRESIMS to be C₁₅H₃₅N₉O. Analysis of the 1D and 2D ¹H and ¹³C NMR data for 1 (CD₃OD, Table 1) revealed resonances and correlations (Figure 1) consistent with those of a fatty acid chain with one terminal methyl (δC 14.2, δH 0.90) and a carbonyl group (δC 175.6). HMBC correlations between C-1 (δC 175.6) and H-2 (δH 2.38), H-17 (1a: δH 3.41; 1b: δH 3.42), and H-21 (δH 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. 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: δH 1.79; 1b: δH 1.89) to H-17 (1a: δH 3.41; 1b: δH 3.42) and H-19 (1a: δH 3.15; 1b: δH 3.21). The butyl chain of the spermidine was determined by COSY correlations from H-21 (1a,b: δH 3.37) to H-22 (1a,b: δH 1.64) and from H-23 (1a,b: δH 1.57) to H-24 (1a,b: δH 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: δC 39.8, δH 3.15; 1b: δC 39.5, δH 3.21) and C-20 (1a,b: δC 2158.4) indicated that the ending methylene of the propyl chain was attached to a guanidine
moiety and the correlation between H-24 (1a, b: δC 41.9, δH 3.21) and C-25 (1a, b: δC 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 C_{47}H_{84}N_{10}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 crambe scidin 800 (6). Two methyl groups C-1 and C-20 (δH 0.87, 1.10; δC 10.9, 21.9), two olefinic carbons C-4 and C-5 (δH 5.52, 5.72; δC 134.4, 131.2), two oxymethines C-3 and C-19 (δH 4.45, 3.87; δC 72.2, 68.3), two N-substituted CH groups C-10 and C-13 (δH 4.06, 4.36; δC 55.4, 53.9), one oxymethylene C-23 (δH 4.14; δC 66.5), two nonprotonated carbons of aminocarbinols C8 and C-15 (δC 82.1, 85.1), a guanidine-like carbon C-21 (δC 150.4), and one ester carbonyl group (δC 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 crambe scidins as indicated by the close similarities of the chemical shifts and coupling constants. On the
basis of the literature, the chemical shift of H-14α 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 isocrambscindine 808, H-14 presented a deshielded chemical shift (δ_H 3.80, d, J = 3.4 Hz). 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 guanidine 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 CH₃), as for crambscindine 800 (6), fromiamycin (9), crambscindic acid (8), and ptilyomycin A (15).

Ptilyomycin F (3) was obtained as a yellow oil. Its molecular formula was established by HRESIMS to be C₄₇H₈₂N₈O₆. A comparison of the ¹H and ¹³C NMR data with those of ptilyomycin 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 H-42 (3a: δ_H 164.0; 3b: δ_C 163.9) as well as between the formamide H-41 (3a: δ_H 8.08; 3b: δ_H 7.98) and C-41 (3a: δ_C 36.5; 3a: δ_C 36.6).

Ptilyomycins 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 (C₄₆H₇₀N₈O₄) was established by HRESIMS. A comparison of the ¹H and ¹³C NMR chemical shifts were determined from the HSQC and HMBC spectra) data with those of ptilyomycin E (2) and ptilyomycin F (3) supported a pantacycliguanidine-like structure with a fatty acid chain. Comparison of the spectroscopic data of the N-terminal fatty acid moiety of ptilyomycin G (4) and ptilyomycin 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_C 3.18; 2b: δ_C 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 (C₄₆H₇₀N₈O₄) 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 ptilyomycin H (5) and ptilyomycin E (2). So differences were observed for the chemical shifts of the butyl group especially for H-46 (5: 2δ_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), ptilyomycins E (2), F (3), the mixture of ptilyomycins G and H (4—5), crambscindine 800 (6), and fromiamycin (9) were evaluated against KB cells and against Plasmodium falciparum (Table 4). Several isolated compounds exhibited strong cytotoxicity, particularly ptilyomycin E (2), the mixture of ptilyomycins G and H (4—5) and fromiamycin (9) with IC₅₀ of 0.85, 0.92, and 1.17 μM,
respectively. As for the antimalarial activity, it seems to be correlated to the pentacyclic moiety; uguiculin A, the acyclic compound showed lower activity (IC$_{50}$ 12.89 $\mu$M) than pentacyclic compounds. Crambescidin 800 (6), fromiamycalin (9), pilomyacin E (2), pilomyacin F (3), and the mixture of pilomyacins G and H (4–5) exhibited IC$_{50}$ of 0.52, 0.24, 0.35, 0.23, and 0.46 $\mu$M, respectively. Lazaro et al. 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 modulation of the activities. The spermidine-containing aliphatic chain of pilomyacin E (2) seems to be more effective for a cytotoxic activity against KB cells and the spermidine-containing aliphatic chain of pilomyacin 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 IC$_{50}$ (KB)/IC$_{50}$ (P. falciparum). As requested by the Medicine for Malaria Venture, several of these compounds present an IC$_{50}$ below the $\mu$M range. Pilomyacin F (3) and fromiamycalin (9) showed similar antimalarial activity, but pilomyacin 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. $^1$H 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 ($\delta$$_{\text{H}}$ 3.31 and $\delta$$_{\text{C}}$ 49.00 for CD$_2$OD). The spectra were processed using 1D and 2D MestReNova (Mnova 10.0 Mestrelab Research) software. HR-ESI MS spectra were recorded using a Waters Acquity BEH C18, 1.7 $\mu$m, 150 × 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 × 26 mm i.d.; 460 × 36 mm i.d. and 230 × 15 mm i.d.) packed with Machery-Nagel MN Kieselgel silica gel (60–200 $\mu$m). 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 C$_{18}$ (150 × 4.6 mm i.d., 3 $\mu$m) column, a Waters Sunfire C$_{18}$ (150 × 4.6 mm i.d., 5 $\mu$m) column, or a Waters Xbridge C$_{18}$ (250 × 4.6 mm i.d., 5 $\mu$m) column and was

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Table 3. $^1$H and $^{13}$C NMR Spectroscopic Data ($^1$H 500 MHz, $^{13}$C 125 MHz, CD$_2$OD) for Pilomyacins G–H (4–5)$^{a,b}$

<table>
<thead>
<tr>
<th>position</th>
<th>$\delta$$_{\text{H}}$ (H)</th>
<th>$\delta$$_{\text{C}}$ (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.7, CH$_3$</td>
<td>0.85, t (7.3)</td>
</tr>
<tr>
<td>2</td>
<td>30.1, CH$_2$</td>
<td>1.46, m</td>
</tr>
<tr>
<td>3</td>
<td>72.1, CH</td>
<td>4.41, br d (10.3)</td>
</tr>
<tr>
<td>4</td>
<td>134.1, CH</td>
<td>5.51, br d (10.9)</td>
</tr>
<tr>
<td>5</td>
<td>131.1, CH</td>
<td>5.71, m</td>
</tr>
<tr>
<td>6</td>
<td>24.3, CH$_2$</td>
<td>2.16, m</td>
</tr>
<tr>
<td>7</td>
<td>38.0, CH$_2$</td>
<td>1.98, m</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>compound</th>
<th>KB cell line IC$_{50}$ $\mu$M</th>
<th>P. falciparum IC$_{50}$ $\mu$M</th>
<th>selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uuguiculin A (1)</td>
<td>7.66 (±0.96)</td>
<td>12.89 (±0.07)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pilomyacin E (2)</td>
<td>0.85 (±0.03)</td>
<td>0.35 (±0.13)</td>
<td>2.4</td>
</tr>
<tr>
<td>Pilomyacin F (3)</td>
<td>1.61 (±0.19)</td>
<td>0.23 (±0.01)</td>
<td>7.0</td>
</tr>
<tr>
<td>Pilomyacin G (4 + 5)</td>
<td>0.92 (±0.05)</td>
<td>0.46 (±0.05)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

---

$^{a,b}$ $^{13}$C NMR chemical shifts were determined from the HSQC and HMBC spectra.
performed on an Agilent 1100 series system controller equipped with a photodiode array detector (Agilent 1100 G1956A) 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) and Waters XBridge C18 Prep (250 × 10 mm i.d., 5 μm), or Phenomenex Gemini C8 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 Poeciloscleridae) was collected in December 2012 in Mitsio Islands, Madagascar (13°39′35″ S, 47°58′21″ 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 CHCl3–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 (35.8 g) was obtained. The extract (35.8 g) was 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). 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 gradient elution with 5% EtOAc–isooxane to 50% EtOAc–isooxane over 20 min; 50% EtOAc–isooxane over 10 min; 50% EtOAc–isooxane to 100% EtOAc over 20 min; 100% EtOAc over 10 min; 0% MeOH–EtOAc to 50% MeOH–EtOAc over 20 min; 50% MeOH–EtOAc to 100% MeOH over 20 min; 100% MeOH over 10 min, gave seven fractions (F31 to F37). Fraction 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 Prep C18, Prep Column, 5 μm, 250 × 10 mm i.d., 4.5 mL/min gradient elution with 2% 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) were collected. Fraction F42 (1.4 mg) was subjected to a subsequent semipreparative HPLC separation (4.5 mL/min gradient elution with 29% CH3CN–H2O (±0.1% formic acid) over 20 min; UV 205 nm) to furnish pure compound 1 (unguiculin A, 1.3 g) and 2 (ptilomycin B, 14 mg).

Fraction F4 (3.16 g) was subjected to preparative HPLC (Waters Sunfire Prep C18, Prep Column, 5 μm, 150 × 19 mm i.d., 18 mL/min 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) to furnish five fractions (F42a to F42e). Fraction F42d was a mixture of 4 and 5 (ptilomycin G and H, 6 mg) and F432e contained the pure compound 2 (ptilomycin 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 gradient elution with 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 (crambescid acid, 1.1 mg).

Fraction F5 (1.51 g) was subjected to preparative HPLC (Waters Sunfire Prep C18, Prep Column, 5 μm, 150 × 19 mm i.d., 18 mL/min 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 to a subsequent semipreparative HPLC (Waters XBridge C18 Prep Column, 5 μm, 250 × 10 mm i.d., 4.7 mL/min 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 (crambescid acid, 800, 12 mg) and 9 (fromiamycin, 2.6 mg).

**Unguiculin A (1).** Yellow oil; [α]D 20° = +18.6 (c 0.9, MeOH). IR (film): νmax 2924, 1626, 1579, 1342, 1015 cm−1; 1H and 13C NMR data, Table 1; HR-ESIMS m/z 468.43820 [M + H]+ (calcd for C31H41O14, 468.43844).

**Ptilomycin E (2).** Yellow oil; [α]D 20° = +3.8 (c 6.9, MeOH). IR (film): νmax 2924, 1626, 1579, 1342, 1015 cm−1; 1H and 13C NMR data, Table 2; HR-ESIMS m/z 689.67041 [M + H]+ (calcd for C46H63N8O5, 689.66989).

**Ptilomycin F (3).** Yellow oil; [α]D 20° = +5.0 (c 2.2, MeOH). IR (film): νmax 2924, 1626, 1579, 1342, 1015 cm−1; 1H and 13C NMR data, Table 3; HR-ESIMS m/z 428.32483 [M + 2H]± (calcd for C34H44N4O4, 428.32314).

**Ptilomycalin G (4) and H (5) (approximately 2:1 mixture).** Yellow oil; [α]D 20° = +3.2 (c 5.3, MeOH). 1H and 13C NMR data, Table 3; HR-ESIMS m/z 827.64819 [M + H]+ (calcd for C48H63N8O5, 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.

**In Vitro Antiplasmodial Assays.** The *P. falciparum* strains utilized and details of the assay protocols have been previously reported.

**Associated Content**

**Supporting Information**

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

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

**Author Information**

**Corresponding Author**

*E-mail: anne.bialecki@univ-reunion.fr.*

**ORCID**

Emerson F. Queiroz: 0000-0001-9567-1664
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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.