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Netamines H–N, Tricyclic Alkaloids from the Marine Sponge *Biemna laboutei* and Their Antimalarial Activity

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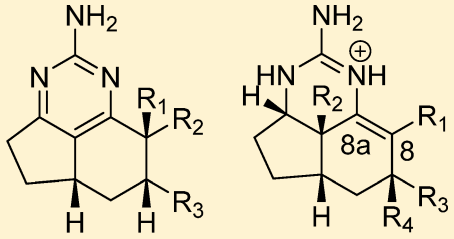
Supporting Information

ABSTRACT: Chemical examination of the CH_2Cl_2–MeOH (1:1) extract of the Madagascar sponge *Biemna laboutei* resulted in the isolation of seven new tricyclic alkaloids, netamines H–N (**1**–**7**), along with the known netamine G and mirabilins A, C, and F. Their structures were elucidated by interpretation of 1D and 2D NMR spectra and HRESIMS data. All compounds were evaluated for their cytotoxicity against KB cells and their antiplasmodial activity. Netamine M (**6**) was found to be cytotoxic, with an IC_{50} value in the micromolar range, and netamine K (**4**) exhibited activity against *Plasmodium falciparum* with an IC_{50} value of 2.4 μM.



Pyrimidine derivative **$\Delta^{8,8a}$ derivative**

Ptilocaulins, mirabilins, and netamines are trivial names representing a rare class of alkaloids possessing a tricyclic (5,6,8*b*)-triazaperhydroacenaphthylene skeleton. These compounds have been exclusively reported from sponges, six of the order Poecilosclerida, namely, *Monanchora arbuscula*[2] and *M. unguifera*,[3,4] *Arenochalina mirabilis*,[5] *Batzella* sp.,[6] *Clathria* sp.,[7,8] and *Biemna laboutei*,[9] and one of the order Axinellida, *Ptilocaulis* aff. *P. spiculifer*.[1] However, re-examination of a voucher specimen of *Ptilocaulis* aff. *spiculifer* (Harbor Branch collection) has led to a re-evaluation of the taxonomy. The voucher specimen seems to fit the poecilosclerid genus *Batzella* Topsent, 1891.[2] The 21 tricyclic alkaloids isolated from the above-mentioned sponges can be grouped on the basis of unsaturation and double-bond positions as pyrimidines or $\Delta^{7,8}$-, $\Delta^{8,8a}$-, $\Delta^{8a,8b}$-, or saturated tricyclic (5,6,8*b*)-triazaperhydroacenaphthylene skeletons.[8] Many of these compounds were reported to have noteworthy biological activities including cytotoxicity[1,4,8,9] and antibacterial,[1,7] antifungal,[3] antimalarial,[3] and antiprotozoal activity.[3]

In our continuing search for bioactive metabolites from marine invertebrates, we found the extract of the Poecilosclerid sponge *Biemna laboutei* (Hooper, 1996)[10] to be cytotoxic to KB cells and active against the malaria parasite *Plasmodium falciparum*. A previous study on *B. laboutei* collected on the southeast coast of Madagascar, twice in May 2004 near Sainte-Marie Island, and once in January 2005 at Itampule, resulted in the isolation of seven alkaloids, designated netamines A–G.[9] However, it should be noted that a stereorevision to netamines A, C, E, and G (*cis*- to *trans*-disposed side chains) was suggested in 2008 by Yu et al.[11] Netamines C and D were found to be particularly cytotoxic against lung (A549), colon (HT29), and breast (MDS-MB-231) cancer cells with GI_{50} values in the micromolar range.[9]

Chemical investigation of the extract of a new batch of *B. laboutei* collected in Madagascar at Salary Bay led to the isolation of the known netamine G and mirabilins A, C, and F and seven new tricyclic alkaloids, netamines H–N (**1**–**7**). All seven possess the (5,6,8b)-triazaperhydroacenaphthylene skeleton. Netamines H–J (**1**–**3**) contain a pyrimidine ring, and netamines K–N (**4**–**7**) show a $\Delta^{8,8a}$ double bond. Herein, we describe the isolation, structure elucidation, and biological characterization of these new compounds.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 (96.9% inhibition at 10 μM concentration) and to exhibit promising *in vitro* antiplasmodial activity (IC_{50} = 3.2 μg/mL). The orange-brown gum was subjected to a combination of

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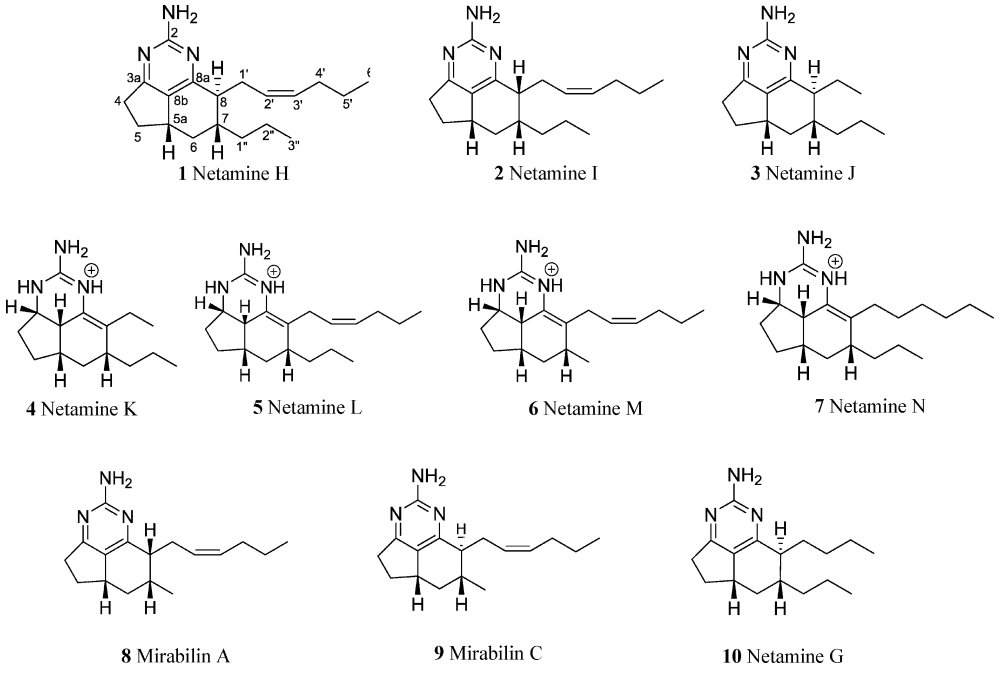
Chart 1



vacuum liquid chromatography over silica gel and repetitive reversed-phase preparative HPLC to yield known and new compounds (**1**–**10**) and mirabilin F. Mirabilins C (**9**) and F as well as netamine G (**10**) were identified by comparison with published spectroscopic data.[8,9]

Netamine H (**1**) was obtained as a brown oil. Its molecular formula was established by HRESIMS to be $C_{19}H_{30}N_3$. The UV spectrum of **1** demonstrated absorption bands at λ_{max} 234 and 303 nm, typical of an aromatic chromophore. Analysis of the 1D and 2D ^{1}H, ^{13}C, and ^{15}N NMR data for **1** (Table 1) revealed resonances and correlations consistent with those of a tricyclic guanidine-like structure with a pyrimidine heterocycle as in mirabilins A (**8**) and C (**9**) and netamine G (**10**): three methylenes (δ 34.2, 34.5, 37.1), three methines (δ_C 38.9, 39.8, 46.5), one trisubstituted double bond (δ_C 127.5, 167.0), an iminium carbon (δ_C 176.5), and a guanidine-like carbon (δ_C 164.6) and three nitrogen atoms (δ_N 78.1, 235.7, 240.5). HMBC correlations for **1** clearly indicated that the heterocyclic ring system is substituted at positions C-7 (δ_C 39.8) and C-8 (δ_C 46.5) by two alkyl groups comprising a total of nine carbons atoms. One chain is a *Z*-hexenyl chain as in mirabilins A and C. The geometry of the double bond was confirmed by an NOE correlation between the two protons H-1′ (δ_H 2.64, 2.78) and H-4′ (δ_H 2.03). The second chain therefore has to be a propyl substituent. The latter propyl group was determined by COSY correlations from H-2″ to H-1″ and H-3″ and confirmed by HMBC correlations from CH_3 (3″) to CH_2 (1″) and CH_2 (2″). The propyl group was found to be attached to C-7 on the basis of COSY correlation from H-1″ to H-7 and HMBC correlation from H-6a to C-1″. The hexenyl group therefore has to be on the C-8 atom. This was confirmed by correlations from H-1′ to C-8 and C-8a. In addition, the two side chains were assigned to be *trans* by conversion of netamine H (**1**) into netamine A. The relative configuration of **1** was indeed established by its hydrogenation into netamine A over Pd/C (3 atm for 4 h) (Scheme 1). The spectroscopic data were in full agreement with those of netamine A,[9] for which the side chains were established to be *trans*.[11]

Table 1. 1D and 2D NMR Spectroscopic Data[a] (CD_3OD) for Netamine H (1)

position	δ_C, type	δ_H (J in Hz)	δ_N	COSY (^{1}H–^{1}H)	HMBC (H–C)
N (1)			240.5[b,c]		
2	164.6, C				
NH_2 (2)		6.45, brs	78.1[b]		
N (3)			235.7[b,c]		
3a	176.5, C				
4	34.5, CH_2	2.56, m		5	3a, 8b
		2.92, m			
5	34.2, CH_2	1.54, m		4, 5a	3a, 8b
		2.39, m			
5a	38.9, CH	2.85, m		5, 6	4
6	37.1, CH_2	0.78, m		5a, 7	8b, 1‴
		2.20, m			
7	39.8, CH	1.79, m		6, 8, 1″	8
8	46.5, CH	2.36, m		7	8a, 6
8a	167.0, C				
8b	127.5, C				
1′	29.4, CH_2	2.64, m		2′	8, 8a
		2.78, m			
2′	126.7, CH	5.18, m		1′, 3′	1′, 4′
3′	133.1, CH	5.39, m		2′, 4′	
4′	30.8, CH_2	2.03, m		3′, 5′	3′
5′	24.0, CH_2	1.33, m		4′, 6′	4′
6′	14.3, CH_3	0.90, t (7.3)		5′	4′, 5′
1″	38.2, CH_2	1.29, m		7, 2″	6
		1.65, m			
2″	21.5, CH_2	1.31, m		1″, 3″	1″
		1.51, m			
3″	14.9, CH_3	0.95, t (7.3)		2″	1″, 2″

[a] ^{1}H chemical shifts were recorded at 500 MHz, ^{13}C chemical shifts were recorded at 125 MHz, ^{15}N chemical shifts were recorded at 60 MHz. [b] Recorded in DMF-d_7. [c] Signals are exchangeable.

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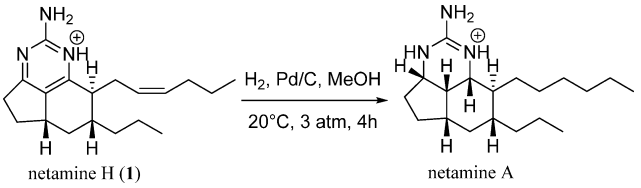
Scheme 1. Reduction of Netamine H (1)



Netamine I (**2**) was isolated as a brown oil, with the same molecular formula, $C_{19}H_{30}N_3$, as for **1**. The ^{13}C NMR data of **2** were very similar to those of **1** except that the signals of C-5a, C-6, C-7, C-8, C-2′, C-3′, and C-1″ shifted from δ_C 38.9, 37.1, 39.8, 46.5, 126.7, 133.1, and 38.2 to 40.4, 33.6, 41.5, 42.9, 131.2, 129.3, and 36.8, respectively. At this stage, detailed analysis of the 2D NMR spectra revealed the planar structure of **2** was identical to the planar structure of netamine H (**1**). The similarity of the NMR spectra of **1** and **2** suggested therefore that these two compounds are stereoisomers. A NOESY experiment on compound **2** recorded in DMF-d_7 showed correlations between H-7 (δ_H 1.94) and both H-5a (δ_H 2.81) and H-8 (δ_H 2.74), confirming they are on the same face. The hexenyl and propyl side chains attached to C-8 and C-7, respectively, were thus established to be *cis*, as opposed to the *trans* configuration in netamine H (**1**). Additionally, as in netamine H (**1**), the geometry of the 2′(3′)-double bond was assigned to be *cis* on the basis of the NOESY correlation, recorded in DMF-d_7, between H-1′ and H-4′.

Netamine J (**3**) was obtained as a pale yellow oil, for which a molecular formula of $C_{15}H_{24}N_3$ was deduced from the HRESIMS and additional spectroscopic data. An excellent correlation between the UV absorptions for **3** and netamines H and I (**1**, **2**), supported a tricyclic guanidine-like arrangement embodying a pyrimidine heterocycle, as did a comparison of their ^{13}C NMR data. The differences observed in the latter ^1H and ^{13}C NMR spectra indicated that the hexenyl group of **1** and **2** is replaced by an ethyl group in **3**. Examination of the COSY data for **3** allowed correlations from methylene CH_2-1′ (δ_H 1.79, 2.13) to the primary methyl CH_3-2′ (δ_H 0.79) and methine CH-8 (δ_H 2.28), thus establishing the C-8 ethyl group. In addition, NOESY correlations recorded in DMF-d_7 positioned H-5a (δ_H 2.81) and H-7 (δ_H 1.70) on a common face and H-8 (δ_H 2.17) on the opposite side of the molecule. The two side chains were therefore established to be *trans*.

Netamine K (**4**) was isolated as a brown oil. HRESIMS data for this compound revealed a molecular formula of $C_{15}H_{26}N_3$, consistent with an ammonium salt with five double-bond equivalents. Analysis of the 1D and 2D ^1H, ^{13}C, and ^{15}N NMR data for **4** revealed resonances and correlations consistent with those of a tricyclic guanidine with a $\Delta^{8,8a}$ double bond, as in mirabilin F, three nitrogen atoms (δ_N 71.0, 87.8, 98.3), three methylenes (δ_C 27.7, 29.5, 37.2), four methines (δ_C 35.5, 36.6, 37.5, 55.0), and three fully substituted sp^2 carbons, which were attributed to a double bond (δ_C 122.7, 129.3) and a guanidine carbon (δ_C 153.4). Additional COSY and HMBC correlations for **4** clearly indicated that the heterocyclic ring system is substituted at positions C-7 (δ_C 36.6) and C-8 (δ_C 129.3) by two alkyl groups, an ethyl group and a propyl group as in netamine J (**3**). That C-7 carries the propyl group (C-1″, -2″, -3″) was confirmed by COSY correlations between H-3″ and H-2″, H-2″ and H-1″, and H-1″ and H-7 and that C-8 carries the ethyl group (C-1′, -2′) were further confirmed by COSY correlation between H-2′ and H-1′ and an HMBC correlation between H-2′ and C-8. Acquisition of the NMR data in DMF-d_7 allowed determination of the relative configuration of the four stereogenic centers: C-3a, C-5a, C-7, and C-8b. The ROESY (DMF-d_7) correlations positioned H-3a, H-5a, H-7, and H-8b on the same face of the molecule.

Netamine L (**5**) was obtained as a brown oil. The molecular formula $C_{19}H_{32}N_3$ was deduced from the HRESIMS and spectroscopic data. A comparison of the ^1H and ^{13}C NMR data with those of netamine K (**4**) supported a tricyclic guanidine including a $\Delta^{8,8a}$ unsaturation and a C-7 propyl chain. The differences observed in the NMR spectra of netamines K (**4**) and L (**5**) indicated that the ethyl group of **4** is replaced by a hexenyl group in **5** as in netamines H (**1**) and I (**2**). Like netamine H (**1**), the *cis* configuration of the side chain double bond was assigned based on an NOE between H-1′ and H-4′. Acquisition of the NMR data in DMF-d_7 aided the determination of the relative configuration of the four asymmetric centers: C-3a, C-5a, C-7, and C-8b. The ROESY (DMF-d_7) correlations positioned clearly and unambiguously H-3a, H-5a, and H-8b on the same face of the molecule. No direct correlation was observed between these three protons and H-7, preventing us from placing H-7 on the same face. However, acquisition of a ^1H NMR spectrum in DMF-d_7 resolved the two H-6 protons (H-6*α*, δ_H 1.66; H-6*β*, δ_H 1.77), and ROESY correlations observed between H-5a and H-6*β* and between H-7 and H-6*β* confirmed occupancy of a common face of the molecule for H-7 and H-5a and thus for H-3a, H-5a, H-7, and H-8b. The observations listed above define the relative configuration for netamine L (**5**) as shown.

Netamine M (**6**) was isolated as a brown oil and had a molecular formula of $C_{17}H_{28}N_3$, which was suggested by HRESIMS. Overall, the NMR features of **6** showed similarity to those of **5** except for the replacement of the propyl group in **5** by a methyl group in **6**. Examination of the COSY spectrum of **6** exhibited correlations between the primary methyl H-1″ (δ_H 1.19) and the methine H-7 (δ_H 2.36), thus establishing the C-7 position of the methyl group. Like netamine L (**5**), the *cis* configuration of the double bond in the hexenyl side chain linked to C-8 was assigned based on an NOE between H-1′ and H-4′. The relative configuration of **6** was determined from NOE enhancements observed in a NOESY experiment performed in acidified DMF-d_7, which in this case improved the resolution of the NOE spectrum. NOE correlations between H-3a and H-8b, and between H-5a and H-8b and H-7, suggested that H-3a, H-5a, H-7, and H-8b were all cofacial.

Netamine N (**7**) was isolated as a brown oil and was assigned the molecular composition of $C_{19}H_{34}N_3$. Netamine N, with two protons more than netamine L (**5**), was found to be the 2′(3′)-saturated analogue of **5**. The NOESY correlations between H-3a, H-5a, and H-8b confirmed occupancy of a common face of the molecule for the three protons. No direct correlation was observed between H-5a and H-7, preventing placement of H-7 on the same face. However, NOESY correlations observed between H-5a and H-6*β* and between H-7 and H-6*β* confirmed occupancy of a common face of the molecule for H-7 and H-5a and thus for H-3a, H-5a, H-7, and H-8b.

Mirabilin A (**8**) was isolated and identified for the first time by Barrow et al. as its *N*-acetyl derivative.[5] The extract was derivatized with pyridine and acetic anhydride before starting fractionation to yield six acetyl derivatives: acetylmirabilins A–F. Here we could isolate mirabilin A for the first time as the underivatized natural product, and its structure was confirmed by spectroscopic analysis. The HRESIMS and ^1H and ^{13}C

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Table 2. ^{1}H NMR Spectroscopic Data (500 MHz, CD_3OD) of Netamines I–N (2–7) and Mirabilin A (8)

position	netamine I (2)	netamine J (3)	netamine K (4)	netamine L (5)	netamine M (6)	netamine N (7)	mirabilin A (8)
3a			3.80, m	3.82, m	3.83, m	3.80, m	
4	1.51, m	2.57, m	1.45, m	1.47, m	1.48, m	1.45, m	2.36, m
	2.36, m	2.93, m	1.99, m	2.00, m	2.02, m	1.99, m	
5	2.59, m	1.53, m	1.64, m	1.79, m	1.63, m	1.63, m	1.53, m
	2.59, m	2.40, m	1.77, m	1.79, m	1.77, m	1.78, m	2.59, m
5a	2.90, m	2.90, m	2.52, m	2.53, m	2.53, m	2.52, m	2.92, m
6	1.24, m	0.78, m	1.60, m	1.86, m	1.47, m	1.58, m	1.80, m
	1.87, m	2.21, m	1.90, m		2.00, m	1.91, m	
7	2.02, m	1.79, m	2.21, m	2.19, m	2.36, m	2.19, m	2.38, m
8	2.82, m	2.28, m					2.77, m
8a							
8b			2.60, m	2.62, m	2.62, m	2.62, m	
1′	2.25, m	1.79, m	2.21, m	3.03, m	2.92, m	1.44, m[a]	2.32, m
	2.54, m	2.13, m		2.90, m	2.97, m		2.50, m
2′	5.23, m	0.79, t (7.5)	1.03, t (7.2)	5.24, m	5.23, m	1.29, m[a]	5.22, m
3′	5.24, m			5.50, m	5.49, m	2.17, m[a]	5.24, m
4′	1.89, m			2.10, m	2.11, m	1.34, m	1.92, m
5′	1.28, m			1.44, m	1.43, m	1.34, m	1.27, m
6′	0.86, t (7.4)			0.93, m	0.95, t (7.4)	0.92, m	0.87, m
1″	1.41, m	1.26, m	1.32, m	1.31, m	1.19, d (7.4)	1.32, m	1.15, m
	1.58, m		1.68, m	1.67, m		1.67, m	
2″	1.46, m	1.33, m	1.34, m	1.31, m		1.56, m	
		1.54, m	1.57, m	1.57, m			
3″	0.97, t (7.0)	0.96, t (7.1)	0.96, t (7.0)	0.93, m		0.96, t (7.0)	

[a]Signals are interchangeable.

Table 3. ^{13}C NMR Spectroscopic Data (125 MHz, CD_3OD) of Netamines I–N (2–7) and Mirabilin A (8)

position	netamine I (2)	netamine J (3)	netamine K (4)	netamine L (5)	netamine M (6)	netamine N (7)	mirabilin A (8)
2	164.4	164.7	153.4	153.4	154.8	153.2	164.5
3a	176.4	176.3	55.0	55.1	55.1	55.1	176.4
4	34.7	34.4	33.2	33.2	33.1	33.2	34.8
5	34.8	34.2	27.7	27.6	28.0	27.9	34.7
5a	40.4	38.8	35.5	35.5	35.6	35.6	40.3
6	33.6	37.4	29.5	28.8	34.6	29.9	35.6
7	41.5	39.4	36.6	37.0	31.7	36.8	34.8
8	42.9	47.2	129.3	127.0	127.2	128.2	44.3
8a	166.8	167.4	122.7	123.4	123.2	122.9	166.9
8b	126.8	127.5	37.5	37.9	37.6	37.4	126.3
1′	29.0	24.4	22.0	27.4	27.2	29.7	29.0
2′	131.2	9.8	13.6	127.9	127.9	30.7	129.4
3′	129.3			132.8	132.7	28.9	131.2
4′	30.6			30.8	30.7	33.2	30.6
5′	23.9			23.9	23.9	23.9	23.9
6′	14.3			14.4	14.3	14.5	14.3
1″	36.8	38.4	36.5	36.3	21.4	36.6	19.6
2″	21.9	21.4	22.6	22.6		22.6	
3″	14.8	14.9	14.5	14.6		14.6	

NMR data, shown in Tables 2 and 3, support the molecular formula $C_{17}H_{26}N_3$, consistent with seven double-bond equivalents. A comparison with reported NMR data for mirabilin A acetate[5] was consistent with unacetylated mirabilin A. Moreover, key correlations in the NOESY data recorded in DMF-d_7, for **8**, distinguished between the two possible isomers, mirabilin A and mirabilin C (**9**). Namely, correlations observed between H-5a (δ_H 2.84), H-7 (δ_H 2.10), and H-8 (δ_H 2.70) supported the proposed mirabilin A C-7/C-8 relative configuration (*cis*) over that of mirabilin C (*trans*). Regrettably, **8** decomposed during preparation of the acetyl derivative Therefore it was not possible to obtain a final confirmation by comparing ^{1}H and ^{13}C NMR data and $[\alpha]_D$ of acetylated **8** with those given by the literature for mirabilin A acetate. However, on the basis of the structural arguments presented above we nevertheless propose the structure of mirabilin A.

The in vitro activities of all netamines isolated were evaluated against KB cells and *P. falciparum*. Netamine M (**6**) was cytotoxic in the micromolar range, with an IC_{50} value of 1 μM. Netamines H (**1**), I (**2**) K (**4**), N (**7**), and G (**10**) and mirabilins A (**8**), C (**9**), and F were inactive at the same concentration. Insufficient amounts of netamines J (**3**) and L

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(**5**) were available for testing. Among the compounds tested (netamines H (**1**), I (**2**), K (**4**), and N (**7**) and mirabilins A (**8**), C (**9**), and F), netamine K (**4**) and mirabilin A (**8**) exhibited antimalarial activity, with IC_{50} values of 2.4 and 20.7 μM respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a MCP 300 Anton Paar modular circular polarimeter at 25 °C (MeOH, *c* in g/100 mL). UV data were recorded in MeOH on a Varian Cary spectrometer. IR spectra were obtained using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR data were acquired with Bruker UltraShield Avance-300 and -500 and DRX-600 spectrometers. Chemical shifts were referenced using the corresponding solvent signals (δ_H 3.31 and δ_C 49.15 for CD_3OD; δ_H 7.27 and δ_C 77.00 for $CDCl_3$; δ_H 8.03 and δ_C 163.00 for DMF-d_7). The isolated compounds showed a higher solubility in CD_3OD. However, $CDCl_3$ was also used in order to compare chemical shifts with the literature data. DMF-d_7 was chosen for the acquisition of ^{15}N NMR spectra. For practical reasons, NOESY spectra were recorded in the same solvent. The spectra were processed using 1D and 2D NMR Notebook software. HRESIMS spectra were recorded on a LCT Premier XE Micromass spectrometer.

MPLC separations were carried out on a Teledyne Isco CombiFlash Companion with a RediSep prepacked normal-phase (120 g) column and on a Büchi system including two C-605 pumps, a C-615 pump manager, a C-660 fraction collector, and a glass column (36 × 46 mm) packed with Macherey-Nagel MN Kieselgel silica gel (70−230 μm). Precoated TLC sheets of silica gel 60-F_{254} were used, and spots were visualized on the basis of the UV absorbance at 254 nm and by heating silica gel plates sprayed with vanillin−sulfuric acid reagent. Analytical HPLC was carried out using a Kinetex (4.6 × 100 mm, 5 μm) column or a Waters Sunfire (4.6 × 150 mm, 5 μm) column and was performed on a Waters 2695 Alliance system equipped with a photodiode array detector (Waters 996), an evaporative light scattering detector (Waters 2420), and a mass spectrometer (Waters Micromass ZQ 2000). Preparative HPLC was carried out using a Waters Sunfire Prep RP18 (19 × 150 mm, 5 μm) column and was performed on a Waters 600 system controller equipped with a photodiode array detector (Waters 2996). All solvents were analytical or HPLC grade and were used without further purification.

Animal Material. The sponge *Biemna laboutei* (phylum Porifera, class Demospongiae, order Poecilosclerida, family Desmacellidae) was collected in October 2009 at four sampling stations in Salary Bay, Madagascar: Station 1 (22°31′727″ S, 43°13′597″ E, at 18 m depth), Station 2 (22°30′952″ S, 43°12′558″ E, at 25−30 m depth), Station 3 (22°31′988″ S, 43°13′036″ E, at 30 m depth), Station 4 (22°31′822″ S, 43°12′939″ E, at 25−27 m depth). The sponge was identified by one of the authors (J.V.), and seven voucher specimens (MHNM.16242.1, MHNM.16242.2, MHNM.16242.3, MHNM.16242.4, MHNM.16242.5, MHNM.16242.6, MHNM.16242.7) were deposited in the Museum d'Histoire Naturelle de Marseille, Palais Longchamp, 1 Bd Philippon, 13004 Marseille, France. Sponge samples were frozen immediately and kept at −20 °C until processed.

Extraction and Isolation. The freeze-dried sponge (358 g) was chopped into small pieces and extracted exhaustively by maceration with fresh CH_2Cl_2−MeOH (1:1) (3 × 3.5 L, each 24 h) at room temperature. After evaporating the solvents under reduced pressure, an orange, oily residue (87 g) was obtained. The extract was then subjected to MPLC over silica gel, eluting with a combination of isohexane, EtOAc, and MeOH of increasing polarity. Four fractions were obtained: F1 eluted with isohexane−EtOAc (95:5); F2 eluted with isohexane−EtOAc (85:15); F3 eluted with EtOAc; and F4 eluted with EtOAc−MeOH (70:30).

Separation of fraction F4 (4.05 g) by vacuum liquid column chromatography over normal-phase silica gel using a combination of heptane, EtOAc, and MeOH as eluents gave 26 fractions (F4-1 to F4-26). TLC and analytical HPLC (Kinetex 5 μm, 4.6 × 100 mm column; 1 mL min^{-1} gradient elution with 45% MeOH−H_2O (+0.1% formic acid) to 70% MeOH−H_2O (+0.1% formic acid) over 30 min; UV 254 nm, ELS) analyses showed the compounds to be present in fraction F4-18 (100% EtOAc). The latter fraction (91 mg) was subjected to preparative HPLC (Waters Sunfire Prep RP18 5 μm, 19 × 150 mm column; 13 mL min^{-1} gradient elution with 45% MeOH−H_2O (+0.1% formic acid) to 70% MeOH−H_2O (+0.1% formic acid) over 45 min; UV 254 nm) to furnish seven fractions, F4-18a, F4-18b, F4-18d, F4-18f, and F4-18g, containing pure compounds **3** (netamine J, 1.9 mg), **8** (mirabilin A, 3.5 mg), **9** (mirabilin C, 2.1 mg), **2** (netamine I, 13 mg), and **1** (netamine H, 3.6 mg), respectively. F4-18e (4.3 mg) was subjected to a subsequent preparative HPLC separation (17 mL min^{-1} gradient elution with 60% MeOH−H_2O (+0.1% formic acid) to 78% MeOH−H_2O (+0.1% formic acid) over 15 min; UV 254 nm) to give pure compound **10** (netamine G, 2.2 mg).

Fraction F4 (220 mg) was subjected to preparative HPLC (Waters Sunfire Prep RP18 5 μm, 19 × 150 mm column; 17 mL min^{-1} gradient elution with 45% MeOH−H_2O (+0.1% formic acid) to 70% MeOH−H_2O (+0.1% formic acid) over 45 min; UV 254 nm). Thirteen subfractions were collected. Four of them afforded mirabilin F (2.9 mg) and pure compounds **5** (netamine L, 6.7 mg), **6** (netamine M, 15.1 mg), and **7** (netamine N, 3.5 mg). Three other fractions were subjected to a subsequent preparative HPLC separation (17 mL min^{-1} gradient elution with 55% MeOH−H_2O (+0.1% formic acid) to 70% MeOH−H_2O (+0.1% formic acid) over 26 min; UV 254 nm) to give pure compound **4** (netamine K, 4.3 mg).

*Netamine H (**1**):* brown oil; $[\alpha]^{25}_D$ +54.58 (*c* 1.20, MeOH); UV (MeOH) λ_{max} (log ε) 202 (1.00), 234 (1.20), 303 (0.50) nm; IR (dry film) ν_{max} 2956, 2870, 1587 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 300.2448 $[M + H]^+$ (calcd for $C_{19}H_{30}N_3$, 300.2440).

*Netamine I (**2**):* brown oil; $[\alpha]^{25}_D$ +0.47 (*c* 4.06, MeOH); UV (MeOH) λ_{max} (log ε) 202 (1.57), 234 (1.94), 304 (0.72) nm; IR (dry film) ν_{max} 3408, 1570 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 300.2444 $[M + H]^+$ (calcd for $C_{19}H_{30}N_3$, 300.2440).

*Netamine J (**3**):* pale yellow oil; $[\alpha]^{25}_D$ +47.7**4** (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.81), 234 (0.87), 303 (0.31) nm; IR (dry film) ν_{max} 3322, 2954, 1589 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 246.1976 $[M + H]^+$ (calcd for $C_{15}H_{24}N_3$, 246.1970).

*Netamine K (**4**):* brown oil; $[\alpha]^{25}_D$ +45.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 201 (0.97), 232 (1.04) nm; IR (dry film) ν_{max} 1681, 1202 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 248.2119 $[M^+]$ (calcd for $C_{15}H_{26}N_3$, 248.2127).

*Netamine L (**5**):* brown oil; $[\alpha]^{25}_D$ +44.8 (*c* 1.46, MeOH); UV (MeOH) λ_{max} (log ε) 202 (1.42), 232 (1.15) nm; IR (dry film) ν_{max} 3262, 1643 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 302.2592 $[M^+]$ (calcd for $C_{19}H_{32}N_3$, 302.2591).

*Netamine M (**6**):* brown oil; $[\alpha]^{25}_D$ +111.4 (*c* 4.01, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.86), 233 (0.82) nm; IR (dry film) ν_{max} 2979, 1580 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 274.2281 $[M^+]$ (calcd for $C_{17}H_{28}N_3$, 274.2283).

*Netamine N (**7**):* brown oil; $[\alpha]^{25}_D$ +4.3 (*c* 0.56, MeOH); UV (MeOH) λ_{max} (log ε) 201 (1.25), 232 (1.2) nm; IR (dry film) ν_{max} 2931, 1681, 1599 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 2 and 3; HRESIMS *m/z* 304.2768 $[M^+]$ (calcd for $C_{19}H_{34}N_3$, 304.2753).

*Mirabilin A (**8**):* brown oil; $[\alpha]^{25}_D$ +2.9 (*c* 1.30, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.00), 235 (2.40), 301 (1.00) nm; IR (dry film) ν_{max} 2959, 1588 cm^{-1}; ^{1}H and ^{13}C NMR, Tables 2 and 3; HRESIMS *m/z* 272.2132 $[M + H]^+$ (calcd for $C_{17}H_{26}N_3$, 272.2127).

Hydrogenation of 1. Netamine H (4 mg) in MeOH (2 mL) was hydrogenated over 10% Pd/C (10 mg) for 4 h at 3 atm. The solution was filtered through Celite, then concentrated to afford 1 mg of netamine A with ∼80% purity.

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 IC_{50} determinations were performed in duplicate experiments and are expressed as individual values.

In Vitro Antiplasmodial Assays. The *P. falciparum* strains utilized and details of the assay protocols have been previously reported.[12]

■ ASSOCIATED CONTENT

Supporting Information

Copies of NMR spectra (^{1}H, ^{13}C) and additional data (tables of NMR data and UV spectra) are provided. This material 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.

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