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Friomaramide, a Highly Modified Linear Hexapeptide from an Antarctic Sponge, Inhibits *Plasmodium falciparum* Liver-Stage Development

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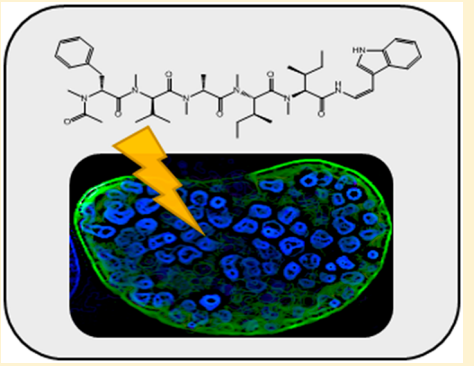
Supporting Information

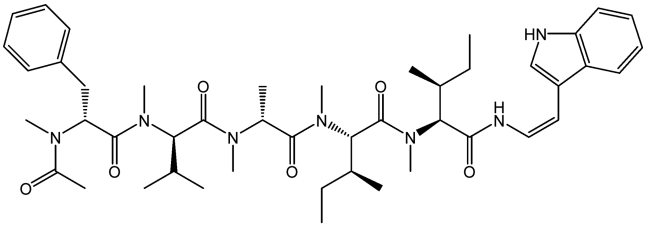
ABSTRACT: The cold waters of Antarctica are known to harbor a rich biodiversity. Our continuing interest in the chemical analysis of Antarctic invertebrates has resulted in the isolation of friomaramide (**1**), a new, highly modified hexapeptide, from the Antarctic sponge *Inflatella coelosphaeroides*. The structure of friomaramide was determined using spectroscopic methods and its configuration established by Marfey's method. Friomaramide, which bears the unusual permethylation of the amino acid backbone and is the longest polypeptide bearing a tryptenamine C-terminus, blocks >90% of *Plasmodium falciparum* liver-stage parasite development at 6.1 μM.



Malaria remains one of the most deadly and widespread infectious diseases, with almost half of the world's population at risk and an estimated 219 million cases reported in 2017.[1] *Plasmodium falciparum* is the most prevalent and lethal cause of malaria, with the highest concentrations in Sub-Saharan Africa.[1] Major successes in global malaria control and elimination have been possible by treating the disease-causing blood stage, but now drug resistance is emerging in the blood-stage infection. Prevention of liver-stage forms of infection, where the parasite numbers are few, represents a vulnerable bottleneck for therapeutic intervention.[2,3] Unfortunately, drug discovery targeting these stages of *Plasmodium* has been limited by the lack of efficient laboratory liver models capable of supporting an affordable medium- to high-throughput drug screening.[4,5] With treatment options scarce, there is a dire need for new lead compounds capable of targeting liver-stage forms of *Plasmodium*.

In search of such malaria treatments, we have screened a collection of natural products from Southern Ocean invertebrates in an *in vitro* liver-stage model.[6] The cold water oceans surrounding Antarctica are geographically and biologically isolated from northern oceans[7] and host a unique, highly productive marine ecosystem.[8] Sponges from these oceans are morphologically and taxonomically diverse and play important ecological roles in benthic ecosystems.[9] Sponges are sessile and soft-bodied and often rely on a high diversity of chemical defenses to deter predation, prevent fouling, and cultivate their extensive symbiotic microorganisms.[10] Sponge natural products therefore remain a promising source of new and unique chemical diversity.

This study of the Antarctic sponge *Inflatella coelosphaeroides* reports the isolation and characterization of a new, highly modified linear peptide, friomaramide (**1**), and its bioactivity in a recently developed *in vitro* liver model of *P. falciparum* infection in metabolically active primary human hepatocytes.[6] Friomaramide is both structurally distinctive and potently bioactive, blocking >90% of *P. falciparum* liver-stage development at 6.1 μM.



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I. coelosphaeroides was collected via trawling in the northern reaches of the Scotia Arc in the Southern Ocean between April and May 2013 (Table S1) at depths of approximately 100−400

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Table 1. NMR Data for Friomaramide in MeOH-d_4

pos	δ_C,[a] type	δ_H,[b] mult. (J in Hz)	gCOSY	gHMBC
Acetyl				
CO	173.9, C			
Me	23.4, CH_3	1.92, s		Ac-CO
N-Me-Phenylalanine				
CO	175.1, C			
α	53.3, CH	5.14, br dd (8.5, 6.7)	β	*N*-Me, Ac-CO, Phe-CO
β	40.2, CH_2	2.90, m	α	γ, δ/δ', Phe-CO
		3.05, m		
γ	141.6, C			
δ/δ'	129.5, CH	7.28, br m	ε/ε', ζ	ζ
ε/ε'	138.5, CH	7.25, br m	δ/δ', ζ	γ
ζ	139.2, CH	7.23, br m	ε/ε', δ/δ'	γ
N-Me	32.6, CH_3	2.86, s		α
N-Me-Valine				
CO	172.7, C			
α	62.9, CH	5.04, d (11.00)	β	γ, γ', *N*-Me, Phe-CO, Val-CO
β	29.8, CH	2.21, m	α, γ, γ'	
γ	12.2, CH_3	0.80, d (7.3)	β	α, γ'
γ'	16.4, CH_3	0.83, d (7.3)	β	α, γ
N-Me	34.6, CH_3	2.78, s		Val-CO
N-Me-Alanine				
CO	175.8, C			
α	59.4, CH	5.17, m	β	*N*-Me, Val-CO, Ala-CO
β	15.7, CH_3	1.20, d (7.0)	α	α, Ala-CO
N-Me	32.8, CH_3	2.95, s		α, Ala-CO
N-Me-Isoleucine$_1$				
CO	172.8, C			
α	59.4, CH	5.09, d (11.0)	β	β-Me, γ, *N*-Me, Ala-CO, Ile$_1$-CO
β	35.7, CH	1.98, m	α, β-Me, γ	
β-Me	19.6, CH_3	0.76, d (6.5)	β	α
γ	26.7, CH_2	1.29, m	δ	α
δ	16.7, CH_3	0.60, d (6.5)	γ	β
N-Me	34.3, CH_3	3.01, s		α, Ile$_1$-CO
N-Me-Isoleucine$_2$				
CO	173.8, C			
α	61.2, CH	5.34, m	β	β-Me, γ, *N*-Me, Ile$_1$-CO, Ile$_2$-CO
β	35.8, CH	2.10, m	α, β-Me, γ	δ
β-Me	16.9, CH_3	0.81, d (6.5)	β	α
γ	26.7, CH_2	1.16, m	δ	α
δ	21.2, CH_3	0.78, d (6.5)	γ	β
N-Me	31.7, CH_3	3.09, s		α, Ile$_2$-CO
Tryptenamine				
1	129.3, CH	6.59, d (8.2)	2	2, 2′, 3a′
2	133.5, CH	6.31, d (8.2)	1	1, 3′
2′	138.0, CH	7.22, s		3′, 3a′, 7a′
3′	109.9, C			
3a′	129.5, C			
4′	128.7, CH	7.60, d (7.8)	5′	3′, 6′, 7a′
5′	130.9, CH	7.08, t (7.2)	4′, 6′	3a′, 7′
6′	132.3, CH	7.15, t (7.2)	5′, 7′	4′, 7′a
7′	121.9, CH	7.36, d (7.8)	6′	3a′, 5′
7a′	138.5, C			

[a] ^{13}C NMR spectrum recorded at 200 MHz. [b] ^{1}H NMR spectrum recorded at 800 MHz, reported in ppm.

m. Sponges were freeze-dried and extracted with CH_2Cl_2/MeOH (1:1). The extract was mounted on silica gel and subjected to normal-phase MPLC. Further NMR-guided purification of the EtOAc/MeOH (3:1) eluting fractions was accomplished with reversed-phase HPLC eluted with MeCN, yielding 1.5 mg of friomaramide (**1**) as an off-white, amorphous solid.

Friomaramide was determined to have a molecular formula of $C_{46}H_{67}N_7O_6$ based on HRESIMS, corroborated by ^{1}H and ^{13}C NMR data (Table 1). Evaluation of the NMR data, as well as the correlations of protons to carbons in the gHSQC spectrum, indicated the presence of five notable nitrogen-bearing methyl groups, multiple aromatic signals, and six ester/amide-type carbonyls, as well as two olefinic, eight methine, three methylene, and eight aliphatic/acyl methyl carbons. These initial observations were suggestive of a highly methylated peptide.

The planar structure of **1** was determined spectroscopically. Among the evident intact amino acid residues, gHMBC data correlated each α-methine proton (δ_H 5.09−5.34) to two amide-type carbonyls (δ_C 172.7−175.8), as well as a singlet *N*-methyl (δ_H 2.78−3.01). These correlations suggested that the molecule was an *N*-methylated peptide. Interestingly, all five of the intact amino acids displayed nitrogen methylation. With this knowledge, the gHMBC and gCOSY spectra were used to construct each amino acid within the molecule (Table 1, Figure 1). Alanine, phenylalanine, valine, and two isoleucine residues, all methylated, were identified.

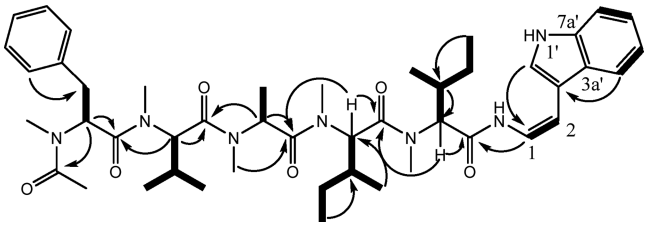

Figure 1. Important gCOSY (bold) and gHMBC (→) correlations establishing the planar structure of friomaramide.

The sequence of the five methylated amino acids was established using gHMBC correlations. In particular, the α-methine protons, which displayed correlations to the adjacent amide carbonyls, were critical to establishing the peptide sequence. The α-methine of *N*-Me-Phe (δ_H 5.14) displayed an HMBC correlation to the *N*-Me-Phe amide carbonyl (δ_C 175.1), but no other amino acid carbonyl, indicating it was the terminal amino acid. The α-methine proton of *N*-Me-Val (δ_H 5.04) displayed correlations to both the *N*-Me-Val (δ_C 172.7) and *N*-Me-Phe carbonyls; the *N*-Me-Ala α-methine (δ_H 5.17) displayed correlations to the *N*-Me-Ala (δ_C 175.8) and *N*-Me-Val carbonyls; the *N*-Me-Ile$_1$ α-methine (δ_H 5.09) displayed correlations to the *N*-Me-Ile$_1$ (δ_C 172.8) and *N*-Me-

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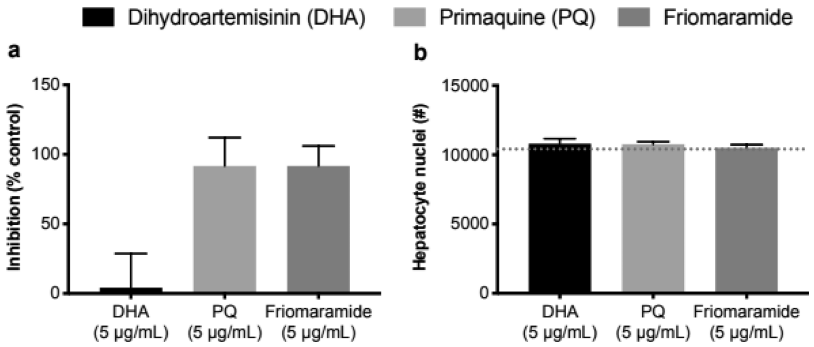

Figure 2. Friomaramide evaluated against *P. falciparum* liver-stage parasites and assessment of hepatocyte toxicity. (a) Blood-stage active antimalarial dihydroartemisinin (DHA) shows no inhibition of *P. falciparum* liver-stage development at 5 μg/mL (17.6 μM), while friomaramide and primaquine (PQ) show >90% inhibition at 5 μg/mL (19.3 and 6.1 μM, respectively). Graph bars represent means with SD for biological replicates (n = 2) and experimental replicates (n = 3). (b) Friomaramide is nontoxic toward primary human hepatocytes at a 5 μg/mL concentration (6.1 μM), where the dotted red line indicates mean hepatocyte nuclei counts for 0.1% DMSO control, comparable to DHA and PQ at the same concentration.

Ala carbonyls; and finally the α-methine of *N*-Me-Ile$_2$ (δ_H 5.34) displayed correlations to the *N*-Me-Ile$_2$ (δ_C 173.8) and *N*-Me-Ile$_1$ carbonyls. The sequence of amino acids was thus established as *N*-Me-Phe-*N*-Me-Ala-*N*-Me-Val-*N*-Me-Ile$_1$-*N*-Me-Ile$_2$.

The N-terminus of the peptide was determined to be acetylated, indicated by gHMBC correlation between the α-methine proton of *N*-Me-Phe (δ_H 5.14) and an acetyl carbonyl at δ_C 173.9.

The C-terminus of friomaramide was found to bear an eliminative decarboxylation product of tryptophan, i.e., tryptenamine. The tryptenamine residue could be constructed by observation of gCOSY-coupled aromatic protons that indicated the presence of an aromatic ring within the residue (δ_H 7.08−7.60). The chemical shift of C-3a′ (δ_C 129.5) and C-7a′ (δ_C 138.5), as well as gHMBC correlations of H-4′ (δ_H 7.60) and H-2′ (δ_H 7.22) with C-3′ (δ_C 109.9) and C-7a′, respectively, verified the fused aromatic ring system of an indole. The olefin comprising positions 1 (δ_C 129.3) and 2 (δ_C 133.5) were identified by gHMBC correlations between C-1 and C-2 with the H-2 and H-1, respectively, as well as the four-bond correlation between H-2′ and C-2. The *cis*-orientation of the olefin was assigned on the basis of $J_{1,2}$ = 8.2 Hz as opposed to the much higher *J*-coupling seen in *trans*-orientation tryptenamine olefins.[11,12] Finally, correlation between the tryptenamine H-2 and the carbonyl of *N*-Me-Ile$_2$ secured its placement on the C-terminus of friomaramide.

Stereochemical analysis of friomaramide was performed by Marfey's method.[13] Briefly, the peptide was hydrolyzed then derivatized with Marfey's reagent, FDAA (N_α-(2,4-dinitro-5-fluorophenyl-L-alaninamide), which allowed the amino acid isomers to be distinguishable from one another chromatographically. These reaction products were evaluated using LC-MS, by comparison to similarly derivatized amino acid standards (Table S2). The absolute configuration of friomaramide was determined to be D-*N*-Me-Phe-L-*N*-Me-Val-L-*N*-Me-Ala-L-*N*-Me-Ile-L-*N*-Me-Ile.

Bioactivity of friomaramide was evaluated for inhibition of *P. falciparum* liver cell infection and development in an *in vitro* liver model,[6] while being simultaneously measured for toxicity against the primary human hepatocytes. Cryopreserved primary human hepatocytes were seeded at 1.8 × 10^4 cells per well in a 384-well microplate. Freshly isolated *P. falciparum* sporozoites were inoculated at 2.0 × 10^4 per well with subsequent drug treatment at 5 μg/mL (17.6, 19.3, and 6.1 μM concentration of dihydroartemisinin, primaquine, and **1**, respectively). Media was changed daily with fresh addition of drug for 3 days until chemical fixation on day 6. Friomaramide showed 92% (±14% standard deviation) inhibition of *P. falciparum* liver-stage infection and development, which is highly comparable to primaquine, one of the few known liver-stage acting antimalarial drugs (Figure 2a). Further, treatment with friomaramide elicited no toxic effects toward primary human hepatocytes determined by quantification of fluorescently labeled hepatocyte nuclei, indicating viable nuclei (Figure 2b). These results identify friomaramide as an important new chemotype that can block infection and development in human liver cells of *P. falciparum*, the most prevalent and lethal cause of malaria.

Friomaramide brings a number of new structural features to light. Tryptenamine as a C-terminus to a small peptide is found only rarely in natural products. The terpeptins, terrestrial fungal natural products, are prenylated tripeptides terminating in tryptenamine.[14] From the marine environment, a marine *Aspergillus* produces terpeptin-like aspergillamides,[15] while the only compounds bearing tryptenamine from macroorganisms are from red algae[11,16] and a tunicate,[17] though in those two examples, the tryptenamine was not part of a peptide chain. Friomaramide introduces a sponge source of this unusual amino acid derivative and, taken with the peptidic nature of the molecule, suggests that host-associated fungi may be the actual producer. Few fungal tryptenamine C-terminating peptides are fully *N*-methylated, although some small peptides lacking tryptenamine are as highly *N*-methylated as friomaramide.[18,19] Those highly methylated peptides that do not bear tryptenamine can be up to eight amino acids in length, while those bearing tryptenamine are never larger than three amino acids. The six amino acid friomaramide appears to be a hybrid between the terpeptin[14,20]/miyakamide[21] type tripeptides and larger peptides such as pterulamide[22] or RHM-type[18] products. Acetylation on the N-terminus amino acid as found in friomaramide is rare among fungal products. Peptide methylation is attractive in drug discovery, as non-methylated peptides have poor pharmacokinetic properties (short *in vivo* half-life, poor oral availability).[23]

In summary, friomaramide is a new, highly modified peptide with potent activity against the liver stage of malaria. In the face of rising antimalarial drug resistance emerging to

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artemisinin combination therapies, the frontline antimalarial drugs of choice, it is important to find new chemodiversity sources. Friomaramide was found to block *P. falciparum* sporozoite infection and subsequent liver-stage parasite development, showing similar inhibitory activity to the known liver-stage antimalarial drug primaquine. Structurally, friomaramide contains a number of unique and uncommon modifications for a small peptide. This chemical novelty and biological specificity present a new framework on which a hit to lead developmental strategy could be expected to bring forward new candidates for malaria treatment.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using an AutoPol IV polarimeter at 589 nm. UV absorptions were measured by an Agilent Cary 60 UV−vis spectrophotometer; IR spectra were recorded with a PerkinElmer Spectrum Two equipped with a UATR (single reflection diamond) sample introduction system. NMR spectra were recorded at 298 K on Varian Direct Drive 800 MHz NMR spectrometers. Chemical shifts are reported with the use of the residual MeOH-d_4 signals (δ_H 3.31 ppm; δ_C 49.0 ppm) as internal standards for ^{1}H and ^{13}C NMR spectra, respectively. The ^{1}H and ^{13}C NMR assignments were supported by gCOSY, gHSQC, and gHMBC experiments. The high-resolution electrospray ionization mass spectra were performed on an Agilent 6230 TOF LC/MS. Medium-pressure liquid chromatography (MPLC) was performed using a Combiflash Rf 200i MPLC, using ELSD and UV detection with a RediSepRf 80 g silica column. Reversed-phase HPLC was completed with a gradient of H_2O to MeOH on a semipreparative Phenominex C18 column (10 μm, 100 Å, 250 × 10 mm) or with a gradient of H_2O to either ACN or MeOH on an analytical Phenominex C18 column (5 μm, 100 Å, 250 × 4.6 mm) using ELSD and UV detection.

Biological Material. Sponge specimens were collected aboard the R/V *Nathaniel B. Palmer* research vessel by trawl and immediately frozen, with tissue samples taken for DNA analyses. Specimens of the sponge *Inflatella coelosphaeroides* Koltun, 1964, include Scripps Institution of Oceanography Benthic Invertebrate Collection accession numbers BIC-SIO S20232, S20400, S20399, S20411, S20437, S20454, and S20375 (see Table S1). Where possible, individual sponges had their DNA extracted using a Qiagen DNeasy kit using the manufacturer's protocol. The DNA was used in a PCR reaction using the forward primer SpongeCOI-F1 but without the M13 tail[24] and the reverse primer dgHCO[25] with an annealing temperature of 40 °C for the first 7 cycles and 50 °C for the subsequent 35 cycles. Amplicons were sequenced at the Australian Genome Research Facility and assembled and edited in Geneious v9.1.8 (Biomatters Ltd.). These sequences were then blasted to the NCBI database (megablast algorithm), with all sequences showing high percentage matches (>99%) to a specimen of *I. coelosphaeroides* collected from the Ross Sea.[24] Two specimens, S20232 and S20399, externally look similar to *I. coelosphaeroides* but have some minor morphological details that differ. Since their LC/MS spectrometric profiles matched with the remaining *I. coelosphaeroides* samples, all were combined for mass extraction. Sequences were deposited in the NCBI database with accession numbers MH892414-MH892417.

Extraction and Isolation. The collection of sponges were extracted in 1:1 CH_2Cl_2/MeOH for two consecutive 24 h periods, immediately followed by extraction in 1:1 MeOH/H_2O for another 24 h. The 1:1 CH_2Cl_2/MeOH extract was dried, and the 6.4 g extract was mounted on silica gel and subjected to NP MPLC with an elution gradient of hexanes to EtOAc, followed by a 1:3 MeOH/EtOAc wash on an 8 g silica column. Fraction E eluted in 1:3 MeOH/EtOAc, displayed ^{1}H NMR signals indicative of the methylated peptide, and was selected for further purification. Semipreparative RP HPLC was performed on fraction E with an elution gradient of 1:3 MeOH/H_2O to MeOH over 40 min, yielding four fractions. One of these fractions, eluting with MeOH, was subjected to analytical RP HPLC with an elution gradient of 3:1 MeCN/H_2O to yield the purified friomaramide (1.5 mg).

*Friomaramide (**1**):* white, amorphous solid; $[\alpha]^{25}_D$ −150 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (2.81); IR (thin film) 3450, 3050, 2980, 2960, 1700, 1680, 1635, 1500, 1240 cm^{-1}; ^{1}H NMR (800 MHz, MeOH-d_4), Table 1; ^{13}C NMR (200 MHz, MeOH-d_4), Table 1; HRESIMS *m*/*z* 814.5226 $[M + H]^+$ (calcd for $C_{46}H_{68}N_7O_6$, 814.5226), *m*/*z* 836.5051 $[M + Na]^+$ (calcd for $C_{46}H_{67}N_7O_6Na$, 836.5045).

Marfey's Analysis. A portion of **1** (0.3 mg) was hydrolyzed at 120 °C with 6 M HCl for 24 h. A 0.1 M $NaHCO_3$ solution (200 μL) was added to the dried hydrolysate of **1**, as well as to L- and D-amino acids standards of *N*-Me valine, *N*-Me alanine, *N*-Me phenylalanine, *N*-Me isoleucine, and *N*-Me *allo*-isoleucine. A solution of Marfey's reagent and FDAA in acetone (25 mg in 50 μL) was added to each hydrolysate and standard, and each was incubated at 90 °C for 10 min. To quench each reaction, 100 μL of 2 M HCl was added and then diluted with 300 μL of MeCN. The Marfey's derivatives of the hydrolysate and standards were analyzed using LC-MS-ToF with a Phemonenex Kinetex C-18 column (2.6 μm, 100 Å, 150 × 3 mm), with an elution gradient of 3:1 H_2O/MeCN acidified with 0.1% formic acid to 1:3 H_2O/MeCN acidified with 0.1% formic acid over 10 min at a 0.4 mL/min flow rate. Comparison of retention time and mass of each Marfey's derivative of the hydrolysate and standard was used to determine the absolute stereochemistry of **1** (Table S2).

Biological Assay. Primary human hepatocytes (PHHs) were plated at 18 000 per well in 40 μL of fresh media on day 0 and were incubated overnight at 37 °C, 5% CO_2. Of the original PHHs plated, roughly 10 000 adhere to the plate. *P. falciparum* sporozoites were added to each well on day 3 postseed of hepatocytes and allowed to incubate for 24 h at 37 °C, 5% CO_2. On days 0, 1, 2, and 3 postsporozoite infection, media was replaced with 40 μL of fresh media, with simultaneous drug administration at a 40 nL volume of a 5 mg/mL drug using a pin tool (V & P Scientific). The pin tool performs a 1000-fold dilution, thus making the final drug concentration 5 μg/mL. The drug-treated media administered on day 3 was removed on day 4 and replaced with fresh media with no further drug administration. On day 6, the media was removed, and the PHHs were chemically fixed using 4% paraformaldehyde, then immunofluorescent staining was performed for quantification and analysis of parasite infection as previously described.[6] This mode is termed "prophylactic", where it is measuring the drugs ability to block sporozoite invasion and subsequent liver-stage parasite development.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00362.

1D ^{1}H and ^{13}C, 2D gCOSY, gHSQC, and gHMBC NMR spectra and HRESIMS for friomaramide; data table of sponge accession numbers and photodocumentation of specimens; data table of Marfey's analysis (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) World Health Organization. World Malaria Report. https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/.
(2) Lindner, S. E.; Miller, J. L.; Kappe, S. H. I. *Cell. Microbiol.* **2012**, *14*, 316−324.
(3) Rabinovich, R. N.; Drakeley, C.; Djimde, A.; Hall, B. F.; Hay, S. I.; Hemingway, J.; Kaslow, D. C.; Noor, A.; Okumu, F.; Steketee, R.; Tanner, M.; Wells, T. N. C.; Whittaker, M. A.; Winzeler, E. A.; Wirth, D. F.; Whitfield, K.; Alonso, P. L. *PLOS Med.* **2017**, *14*, e1002456.
(4) Campo, B.; Vandal, O.; Wesche, D. L.; Burrows, J. N. *Pathog. Global Health* **2015**, *109*, 107−122.
(5) Baird, K. *Pathog. Global Health* **2015**, *109*, 93−106.
(6) Roth, A.; Maher, S. P.; Conway, A. J.; Ubalee, R.; Chaumeau, V.; Andolina, C.; Kaba, S. A.; Vantaux, A.; Bakowski, M. A.; Luque, R. T.; Adapa, S. R.; Singh, N.; Barnes, S. J.; Cooper, C. A.; Rouillier, M.; McNamara, C. W.; Mikolajczak, S. A.; Sather, N.; Witkowski, B.; Campo, B.; Kappe, S. H. I.; Lanar, D. E.; Nosten, F.; Davidson, S.; Jiang, R. H. Y.; Kyle, D. E.; Adams, J. H. *Nat. Commun.* **2018**, *9*, 1837.
(7) McClintock, J. B.; Amsler, C. D.; Baker, B. J. *Integr. Comp. Biol.* **2010**, *50*, 967−980.
(8) Clarke, A.; Murphy, E. J.; Meredith, M. P.; King, J. C.; Peck, L. S.; Barnes, D. K. A.; Smith, R. C. *Philos. Trans. R. Soc., B* **2007**, *367*, 149−166.
(9) Hooper, J. N. A.; Van Soest, R. W. M. *Systema Porifera*; Springer: Boston, MA, 2002.
(10) von Salm, J. L.; Schoenrock, K. M.; McClintock, J. B.; Amsler, C. D.; Baker, B. J. The status of marine chemical ecology in Antarctica: form and function of unique high-latitude chemistry. In *Marine Chemical Ecology*; Puglisi, M. P.; Becerro, M. A., Eds.; CRC Press: Boca Raton, FL, 2019; pp 27−60.
(11) Palermo, J. A.; Flower, P. B.; Seldes, A. M. *Tetrahedron Lett.* **1992**, *33*, 3097−3100.
(12) Ondeyka, J. G.; Dombrowski, A. W.; Polishook, J. P.; Felcetto, T.; Shoop, W. L.; Guan, Z.; Singh, S. B. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 220−224.
(13) Bhushan, R.; Brückner, H. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 3148−3161.
(14) Kagamizono, T.; Sakai, N.; Arai, K.; Kobinata, K.; Osada, H. *Tetrahedron Lett.* **1997**, *38*, 1223−1226.
(15) Toske, S.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Tetrahedron* **1998**, *54*, 13459−13466.
(16) Kirkup, M. P.; Moore, R. E. *Tetrahedron Lett.* **1983**, *24*, 2087−2090.
(17) Appleton, D. R.; Page, M. J.; Lambert, G.; Berridge, M. V.; Copp, B. R. *J. Org. Chem.* **2002**, *67*, 5402−5404.
(18) Boot, C. M.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2006**, *69*, 83−92.
(19) Boot, C. M.; Amagata, T.; Tenney, K.; Compton, J. E.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *Tetrahedron* **2007**, *63*, 9903−9914.
(20) Izumikawa, M.; Hashimoto, J.; Takagi, M.; Shin-Ya, K. *J. Antibiot.* **2010**, *63*, 389−391.
(21) Shiomi, K.; Hatae, K.; Yamaguchi, Y.; Masuma, R.; Tomoda, H.; Kobayashi, S.; Omura, S. *J. Antibiot.* **2002**, *55*, 952−961.
(22) Lang, G.; Mitova, M. I.; Cole, A. L.; Din, L. B.; Vikineswary, S.; Abdullah, N.; Blunt, J. W.; Munro, M. H. *J. Nat. Prod.* **2006**, *69*, 1389−1393.
(23) Chatterjee, J.; Gilon, C.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331−1342.
(24) Vargas, S.; Kelly, M.; Schnabel, K.; Mills, S.; Bowden, D.; Wörheide, G. *PLoS One* **2015**, *10*, 0127573.
(25) Meyer, C.; Geller, J.; Paulay, G. *Evolution* **2005**, *59*, 113−125.

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