

## A New Bioactive Sesterterpene and Antiplasmodial Alkaloids from the Marine Sponge *Hyrtios cf. erecta*

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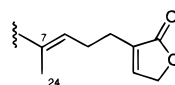
From the CH<sub>2</sub>Cl<sub>2</sub> extract of the sponge *Hyrtios cf. erecta*, collected from Fiji, two new sesterterpenes, **1** and **2**, and the known compounds isodehydroloffariellolide (**3**), homofascaplysin A (**4**), and fascaplysin (**5**) were isolated. The structures of **1–5** were established employing 1D and 2D NMR spectroscopy and mass spectrometry. All NMR resonances of fascaplysin (**5**) have been unambiguously assigned. Evaluation of the biological activity of the extracts and pure compounds toward *Plasmodium falciparum*, *Trypanosoma brucei* subsp. *rhodesiense*, *Trypanosoma cruzi*, hepatitis A virus (HAV), several other microbial targets, and HIV-1-RT and p56<sup>lck</sup> tyrosine kinase revealed new activities for homofascaplysin (**4**) and fascaplysin (**5**), both being potently active in vitro against *P. falciparum*.

Sponges are an important source for new marine natural products, as the majority of compounds reported with biomedical or ecological importance have been obtained from this group of organisms.<sup>1</sup> Many biologically active sponge metabolites are alkaloids or terpenes. Compounds that have been proven to be of further interest are antiinflammatory sesterterpenes, for example, manoalide, which is used for studying inflammatory processes.<sup>2,3</sup> From *Hyrtios* sp., a multitude of biologically active and structurally diverse secondary metabolites have been described. The extract of *H. erecta* gave the immunosuppressive hyrtiomanzamines,  $\beta$ -carboline alkaloids.<sup>4</sup> Cytotoxic sesterterpenes with scalarane and the newly described hyrtiosane skeleton have been found in *H. erectus*,<sup>5,6</sup> and althyrines A–C, macrolides with cytotoxic activity in cell cultures, have been isolated from *H. altum*.<sup>7,8</sup> In the course of the biological screening of marine organisms collected from Fiji, a sample of *Hyrtios cf. erecta* (family Thorectidae) attracted our attention because of its activities toward various screening targets in benchtop assays. HIV-1-RT and p56<sup>lck</sup> tyrosine kinase were inhibited by the CH<sub>2</sub>Cl<sub>2</sub> extract of the animal, and the CH<sub>2</sub>Cl<sub>2</sub> and MeOH extracts were found to significantly inhibit the growth of several bacteria and fungi in agar-diffusion assays. In the present work, the isolation and structure elucidation of two new sesterterpene lactones, **1** and **2**, and isodehydroloffariellolide (**3**)<sup>9</sup> are reported, together with the bioassay-guided isolation of homofascaplysin A (**4**)<sup>9</sup> and fascaplysin (**5**).<sup>10</sup> Compounds **3–5** have previously been reported to occur in the genus *Fascaplysinopsis*.<sup>9,10</sup>

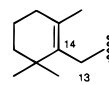
### Results and Discussion

Immediately after collection, sponge material was preserved in EtOH. Prior to extraction, the preservation EtOH was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and water/MeOH. The sponge material was exhaustively extracted with CH<sub>2</sub>Cl<sub>2</sub> followed by MeOH, and the CH<sub>2</sub>Cl<sub>2</sub> and MeOH phases were combined. The CH<sub>2</sub>Cl<sub>2</sub> extract was fractionated using normal-

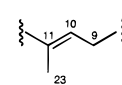
phase vacuum liquid chromatography (VLC). Chemical screening by <sup>1</sup>H NMR spectroscopy showed several fractions to contain terpenoids. Normal-phase HPLC of those fractions led to the isolation of two new sesterterpenes, **1** and **2**, and isodehydroloffariellolide (**3**).<sup>10</sup> Biological screening using HIV-1-RT and p56<sup>lck</sup> tyrosine kinase inhibition assays revealed a further fraction to contain active metabolites. Purification of this fraction by reversed-phase HPLC yielded the alkaloids homofascaplysin A (**4**), which inhibited only p56<sup>lck</sup> tyrosine kinase, and fascaplysin (**5**), a known HIV-1-RT inhibitor that also inhibited p56<sup>lck</sup> tyrosine kinase. Both alkaloids were found to potently inhibit the growth of *Plasmodium falciparum*.



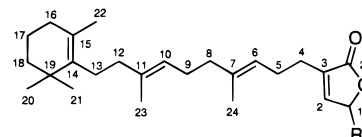
Substructure I of **1**



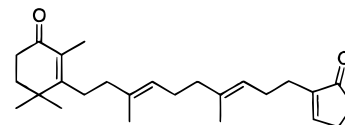
Substructure II of **1**



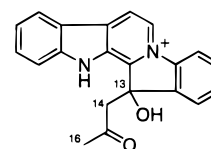
Substructure III of **1**



**1** R=H **2** R= OCH<sub>2</sub>CH<sub>3</sub>



Isodehydroloffariellolide (**3**)



Homofascaplysin A (**4**)

The molecular formula of **1**, C<sub>25</sub>H<sub>38</sub>O<sub>2</sub>, was deduced from accurate mass measurements. Absorption bands in its IR

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**Table 1.**  $^1\text{H}$  NMR Data ( $\delta$ , ppm) for Compounds **1** and **2**

proton	<b>1</b> <sup>a</sup>	$^1\text{H}$ - $^1\text{H}$ COSY <sup>b</sup>	<b>2</b> <sup>c</sup>	$^1\text{H}$ - $^1\text{H}$ COSY <sup>b</sup>
1	4.76 (d, $J = 1.6$ Hz)	H-2, H-4	5.78 (d, $J = 1.5$ Hz)	H-2, H-4
2	7.09 (t, $J = 1.6$ Hz)	H-1, H-4	6.75 (d, $J = 1.5$ Hz)	H-1, H-4
4	2.34 (m)	H-1, H-2, H-5	2.33 (m)	H-1, H-2, H-5, H-6
5	2.27 (m)	H-4, H-24	2.25 (m)	H-4, H-6, H-24
6	5.12 (m)	H-4, H-5, H-24	5.1 (m)	H-4, H-5, H-24
8	2.03 (m)		1.99 (m)	
9	2.07 (m)	H-10	2.06 (m)	H-10, H-23
10	5.12 (m)	H-9, H-23	5.1 (m)	H-9, H-23
12	2.02 (m)		2.01 (m)	
13	2.03 (m)		2.02 (m)	
16	1.90 (dd, $J = 6.2$ Hz)	H-17, H-18	1.89 (dd, $J = 6.1$ Hz)	H-17, H-18
17	1.55 (m)	H-16, H-18	1.55 (m)	H-16, H-18
18	1.41 (m)	H-16, H-17	1.41 (m)	H-16, H-17, H-20, H-21
20	0.99 (s)		0.98 (s)	
21	0.99 (s)		0.98 (s)	
22	1.60 (s)		1.59 (s)	
23	1.64 (s)	H-10	1.63 (s)	H-10
24	1.61 (s)	H-6	1.60 (s)	H-6
26			3.9 (dq, $J = 7.1, 9.2$ Hz)	H-27
			3.72 (dq, $J = 7.1, 9.2$ Hz)	
27			1.27 (t, $J = 7.1$ Hz)	H-26

<sup>a</sup>  $\text{CDCl}_3$ , 300 MHz. <sup>b</sup> Relevant couplings only. <sup>c</sup>  $\text{CDCl}_3$ , 400 MHz.

**Table 2.**  $^{13}\text{C}$  NMR Data ( $\delta$ , ppm) for Compounds **1** and **2**

carbon	<b>1</b> <sup>a</sup>	diagnostic HMBC	<b>2</b> <sup>b</sup>	diagnostic HMBC
1	70.1, t <sup>c</sup>		101.5, d	
2	144.2, d		142.2, d	
3	134.0, s	H-4, H-5	138.1, s	H-1, H-2, H-4, H-5
4	25.5, t		25.5, t	
5	25.7, t		25.4, t	
6	123.4, d		122.4, d	
7	136.8, s	H-5, H-8, H-24	137.0, s	H-5, H-8, H-24
8	39.7, t	H-6, H-9, H-24	39.7, t	H-9, H-24
9	26.6, t	H-8, H-10	26.6, t	H-8, H-10, H-23
10	122.7, d	H-9, H-12, H-23	123.4, d	H-9, H-23
11	136.2, s	H-9, H-12, H-23	136.2, s	H-9, H-12, H-23
12	40.3, t	H-10, H-13, H-23	40.3, t	H-13, H-23
13	28.0, t	H-12	28.0, t	H-12
14	137.1, s	H-13, H-20, H-21, H-22	137.2, s	H-13, H-20, H-21, H-22
15	126.9, s	H-13, H-16, H-22	126.9, s	H-13, H-16, H-22
16	32.8, t		32.8, t	
17	19.5, t		19.6, t	
18	39.9, t		39.9, t	
19	35.0, s	H-18, H-20, H-21	35.0, s	H-18, H-20, H-21
20	28.6, q	H-18, H-21, H-22	28.6, q	H-18, H-21, H-22
21	28.6, q	H-18, H-20, H-22	28.6, q	H-18, H-20, H-22
22	19.8, q	H-16	19.8, q	
23	16.0, q	H-10, H-12	16.0, q	H-10
24	16.1, q	H-6, H-8	16.1, q	H-6, H-8
25	174.4, s	H-1, H-2, H-4	171.5, s	H-1, H-2, H-4
26			65.8, t	H-1, H-27
27			15.1, q	H-26

<sup>a</sup>  $\text{CDCl}_3$ , 75.5 MHz. <sup>b</sup>  $\text{CDCl}_3$ , 100.6 MHz. <sup>c</sup> Multiplicity by DEPT (s = C, d = CH, t =  $\text{CH}_2$ , q =  $\text{CH}_3$ ).

spectrum were characteristic of an  $\alpha,\beta$ -unsaturated lactone ( $\nu_{\text{max}}$  1755, 1345  $\text{cm}^{-1}$ ). The  $^{13}\text{C}$  NMR spectrum of **1** contained one signal for an ester carbonyl carbon,  $\delta$  174.4 (s), five signals for fully substituted olefinic carbon atoms ( $\delta$  137.1 s, 136.8 s, 136.2 s, 134.0 s, 126.9 s), and three signals for CH olefinic carbon atoms ( $\delta$  144.2 d, 123.4 d, 122.7 d). From these data and the  $^1\text{H}$  NMR data (see Table 1) it was concluded that the molecule had four carbon-carbon double bonds and an ester carbonyl carbon as the only multiple bonds; the molecule is bicyclic. Further evidence for the ester being an  $\alpha,\beta$ -unsaturated lactone functionality came from signals in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra assigned to C-1, C-2, and C-3 (Table 1). Comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Tables 1 and 2) with published data revealed **1** to possibly be a sesterterpene with one end of the molecule similar to isodehydroluffariellolide (**3**), substructure I, and the other resembling manoalide.<sup>3</sup> Thus, substructure I, including C-1, C-2, and C-25, and substructure II, from C-14 to C-22, could be assigned. The  $\beta$ -sub-

stituted butenolide (substructure I) moiety could be confirmed by interpretation of the  $^1\text{H}$ - $^1\text{H}$  COSY and  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectra. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** revealed a spin system from H<sub>2</sub>-1 through to H<sub>3</sub>-24 consistent with the proposed substructure I (Table 1). Further,  $^1\text{H}$ - $^{13}\text{C}$  HMBC correlations positioned the quaternary carbons C-3, C-7, and C-25 in substructure I (Table 2). Cross-peaks in the  $^1\text{H}$ - $^1\text{H}$  COSY between H<sub>2</sub>-16, H<sub>2</sub>-17, and H<sub>2</sub>-18 and  $^1\text{H}$ - $^{13}\text{C}$  HMBC couplings observed from C-14, C-15, C-19, C-20, C-21, and C-22 to the relevant protons (Table 2) proved substructure II from C-13 to C-22.  $^1\text{H}$ - $^1\text{H}$  couplings in the COSY between H<sub>2</sub>-9, H-10, and H<sub>3</sub>-23 revealed the presence of a further isoprenoid moiety from C-9 to C-23 (substructure III). The three substructures accounted for all atoms within **1**, apart from two methylene groups;  $\text{CH}_2$ -8 and  $\text{CH}_2$ -12. The connection of fragments I to III and positions of C-8 and C-12 were achieved by assigning the  $^1\text{H}$ - $^{13}\text{C}$  long-range couplings between C-7, C-9, C-11, and C-12 and the respective protons (Table 2) to give **1**.

**Table 3.**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75.5 MHz) Data for Fascaplysin (**5**)

carbon	$^1\text{H}$ , $\delta$ , ppm	$^{13}\text{C}$ , $\delta$ , ppm	ROESY	diagnostic HMBC
1a		126.2, s <sup>a</sup>		H-2, H-3, H-4
1	8.11 (dd, $J = 1.3, 7.5$ Hz)	127.6, d	H-2	
2	7.80 (dd, $J = 0.9, 7.5$ Hz)	133.5, d	H-1, H-3	
3	8.03 (dd, $J = 1.3, 8.0$ Hz)	139.2, d	H-2, H-4	
4	8.37 (d, $J = 8.0$ Hz)	117.3, d	H-3, H-6	
4a		149.4, s		H-1, H-2, H-3, H-4, H-6
6	9.41 (d, $J = 6.2$ Hz)	128.4, d	H-4, H-7	
7	9.00 (d, $J = 6.2$ Hz)	121.9, d	H-6, H-8	
7a		143.6, s		H-6, H-7, H-8, H-9
7b		121.9, s		H-7, H-8, H-9, H-10, H-11
8	8.53 (d, $J = 8.0$ Hz)	125.9, d	H-7, H-9	
9	7.59 (ddd, $J = 0.9, 7.1, 8.0$ Hz)	125.4, d	H-8, H-10	
10	7.94 (ddd, $J = 1.3, 7.1, 8.4$ Hz)	136.7, d	H-9, H-11	
11	7.86 (dd, $J = 0.9, 8.4$ Hz)	115.4, d	H-10	
11a		149.6, s		H-8, H-9, H-10, H-11
12a		133.7, s		H-7
12b		124.1, s		H-6, H-7
13		183.9, s		H-1, H-2, H-4

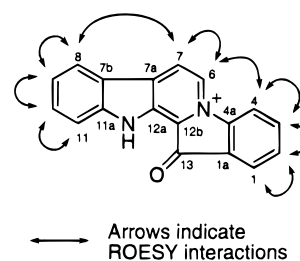
<sup>a</sup> Multiplicity by DEPT ( $s = \text{C}$ ,  $d = \text{CH}$ ,  $t = \text{CH}_2$ ,  $q = \text{CH}_3$ ).

Connectivities deduced from the  $^1\text{H}$ - $^1\text{H}$  COSY and  $^1\text{H}$ - $^{13}\text{C}$  HMBC correlations of **1** were not always unambiguous due to signal overlap.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1**, however, compare favorably with those of isodehydroluffariellolide and manoalide. The  $^{13}\text{C}$  NMR chemical shifts of the resonances for the C-23 ( $\delta$  16.0) and C-24 methyl groups ( $\delta$  16.1) revealed the double bonds  $\Delta^{6,7}$  and  $\Delta^{10,11}$  to have *E* configurations.<sup>11</sup> Compound **1** is thus a new sesterterpenoid for which the trivial name hyrtiolide is proposed.

Compound **2** was analyzed for  $\text{C}_{27}\text{H}_{42}\text{O}_3$  by accurate mass measurement. Significant fragment ions in the EIMS of **2** observed at  $m/z$  414 [ $\text{M}]^+$ ; 369 [ $\text{M} - \text{OC}_2\text{H}_5]^+$ ; and 290, 272, and 137, which were also characteristic of compound **1**, indicated **2** to be a  $-\text{OC}_2\text{H}_5$  derivative of **1**. Additionally, IR bands at 1770 and  $1340\text{ cm}^{-1}$  indicated **2** to contain an  $\alpha,\beta$ -unsaturated lactone moiety. Inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **2** (Tables 1 and 2) revealed it to be essentially identical to that of **1**, except for the resonances assigned to the atoms associated with the  $\alpha,\beta$ -unsaturated lactone and resonances attributable to an ethoxyl moiety ( $\text{CH}_2$ -26,  $\text{CH}_3$ -27, see Tables 1 and 2). The downfield shift of C-1 ( $\delta$  101.5 d) and H-1 ( $\delta$  5.78 d,  $J_{\text{HH}} = 1.5$  Hz) if compared to **1**, revealed C-1 to be substituted with a further oxygen-containing functionality, indicating the ethoxyl group to reside on C-1; this is in accordance with further literature data.<sup>12</sup> This proposition was also supported by all of the remaining NMR data, including  $^1\text{H}$ - $^1\text{H}$  COSY and  $^1\text{H}$ - $^{13}\text{C}$  long-range (HMBC) NMR spectra (Tables 1 and 2). Compound **2** is thus the 1-*O*-ethyl derivative of **1**. As the sponge was preserved in EtOH it is likely, however, that **2** is an artifact of preservation and not a natural product in its own right.

The sample contained several other sesterterpenes, of which isodehydroluffariellolide (**3**) was identified by comparison of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data with published data.<sup>10</sup> Together with the sesterterpenes, the two alkaloids homofascaplysin A (**4**) and fascaplysin (**5**) were also isolated. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data of homofascaplysin A (**4**) are extremely solvent dependent, and, as the chemical shifts in  $\text{CD}_3\text{OD}$  differed markedly from those in  $\text{CDCl}_3$ , they are reported in the Experimental Section. It is also interesting to note that **4** is only sparingly soluble in  $\text{CDCl}_3$  to the point where good NMR data are difficult to obtain. In  $\text{CD}_3\text{OD}$ , however, homofascaplysin A (**4**) is readily soluble and high quality NMR data were obtained in a routine manner.

Fascaplysin (**5**) was identified by FABMS measurements and NMR spectroscopy. The FABMS showed an intense



**Figure 1.** Structure of fascaplysin (**5**) showing all of the observed ROESY correlations.

ion at  $m/z$  271 [ $\text{M}^+$ ] for  $\text{C}_{18}\text{H}_{11}\text{N}_2\text{O}$ , and the  $^1\text{H}$  and  $^{13}\text{C}$  NMR measurements revealed resonances in agreement with literature values (Table 3).<sup>9</sup> As the previously published<sup>9</sup> NMR data did not allow the structure of **5** to be unambiguously assigned, this task was undertaken and yielded complete and unambiguous  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments for fascaplysin (**5**). A  $^1\text{H}$ - $^{13}\text{C}$  HMQC spectrum of **5** led to the correlation of all proton resonances with the resonances of their directly bonded carbon atoms. Positions of all CH signals were deduced from the result of a 2D ROESY experiment, and  $^1\text{H}$ - $^1\text{H}$  couplings allowed the assignment of all resonances from H-1 to H-11 (Figure 1, Table 3). For the assignment of the eight quaternary carbon atoms, a  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum was recorded. Diagnostic long-range correlations between C-1a, C-4a, C-7a, C-7b, C-11a, and C-13 and the respective protons (see Table 3) resulted in the assignment of all but two resonances. The remaining carbon atoms, C-12a and C-12b, could be distinguished from each other first by cross-peaks in the  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum between C-12a and H-7, and C-12b and H-6 and H-7, and second by their chemical shifts,  $\delta$  133.7 being typical for carbons neighboring nitrogen (C-12a), and  $\delta$  124.1 being typical for carbons neighboring a carbonyl and quaternary nitrogen (C-12b), as found in reticulatine<sup>13</sup> and strychnoxanthine.<sup>14</sup>

The isolation of a mixture of alkaloids and sesterterpenes, as described in the current study, is typical for sponges belonging to the family Thorectidae. The finding of **3**-**5** in the genus *Hyrtios* and *Fascaplysinopsis* underlines chemotaxonomically their classification in the same family. It is, however, possible that in the past specimens were identified incorrectly.

**Biological Activity.** During the primary screening of extracts using agar diffusion assays the  $\text{CH}_2\text{Cl}_2$  and MeOH extracts of *Hyrtios* cf. *erecta* were found to inhibit growth

**Table 4.** Biological Activity of Homofascaplysin A (**4**) and Fascaplysin (**5**)

compound	antiplasmodial activity (IC <sub>50</sub> , ng/mL)		cytotoxicity (MIC, µg/mL)	
	K1 <sup>a</sup>	NF54 <sup>b</sup>	L-6 <sup>c</sup>	macrophages <sup>d</sup>
homofascaplysin A ( <b>4</b> )	14 <sup>e</sup>	24 <sup>e</sup>	1.1	30
fascaplysin ( <b>5</b> )	50 <sup>f</sup>	34 <sup>f</sup>	2.5 <sup>e</sup>	
chloroquine	54 <sup>g</sup>	4 <sup>g</sup>		
artemisinin	1 <sup>g</sup>	2 <sup>g</sup>		

<sup>a</sup> *P. falciparum* strain K1. <sup>b</sup> *P. falciparum* strain NF54. <sup>c</sup> Rat skeletal muscle myoblast (L-6) cells. <sup>d</sup> Mouse peritoneal macrophages. <sup>e</sup> Average value, *n* = 2. <sup>f</sup> Average value, *n* = 3. <sup>g</sup> Average value, *n* = 4.

of the bacteria *Escherichia coli* and *Bacillus megaterium* and of the fungi *Ustilago violaceae*, *Eurotium repens*, and *Mycotypha microspora*. The CH<sub>2</sub>Cl<sub>2</sub> extract also inhibited HIV-1-RT and p56<sup>lck</sup> tyrosine kinase in ELISA-based assays. At a concentration of 66 µg/mL it reduced the activity of HIV-1-RT to 15%; at a concentration of 100 µg/mL, the activity of p56<sup>lck</sup> tyrosine kinase to 20%. Of the pure natural products, compound **1** showed weak antifungal activity toward *U. violaceae* (50 µg substance/2-mm growth inhibition zone). Isodehydroluffariellolide (**3**) reduced the activity of p56<sup>lck</sup> tyrosine kinase at 0.5 mM to 45%. Homofascaplysin A (**4**) inhibited the growth of *E. coli* (50 µg/9 mm) and *B. megaterium* (50 µg/11 mm); the activity of p56<sup>lck</sup> tyrosine kinase was reduced to 8% at 0.6 mM, and to 44% at 0.3 mM, with the activity of HIV-1-RT being unaffected. Fascaplysin (**5**) inhibited the growth of *E. coli* (50 µg/6 mm) and *B. megaterium* (50 µg/10 mm); the activity of p56<sup>lck</sup> tyrosine kinase was reduced to 10% at a concentration of 0.7 mM, and that of HIV-1-RT at 0.12 mM to 10% residual activity. Further biological activity for **5** was found against *Trypanosoma brucei* subsp. *rhodesiense*, displaying moderate activity (IC<sub>50</sub> value, 0.17 µg/mL = 630 nM) compared with melarsoprosol (IC<sub>50</sub> value, 2 ng/mL). The evaluation of antiviral activity of fascaplysin (**5**) revealed an increased cytopathogenic effect at noncytotoxic concentrations (0.038 µg/mL) toward fetal Rhesus monkey kidney cells (FRhK-4-cells) infected with the HAV-variant HAVcytHB1.1. Persistently infected FRhK-4-cells (HAV/7) showed cytopathogenicity at 0.038 µg/mL of fascaplysin (**5**), while untreated FRhK-4-cells remained unchanged. Homofascaplysin A (**4**) and fascaplysin (**5**) were shown to be potent in vitro inhibitors of chloroquine-susceptible (NF54) and chloroquine-resistant *P. falciparum* strains (Table 4). Positive control substances were chloroquine and artemisinin. The potency against the K1 strain of homofascaplysin A (**4**) was stronger than that of chloroquine. Compared with artemisinin (K1 strain) and with both positive control substances (NF54 strain), **4** was approximately 10-fold less active. Cytotoxicity against L-6 muscle cells and mouse macrophages was at a level that would indicate these compounds to be unlikely candidates as drugs themselves (Table 4). The potent antiplasmodial activity, however, of **4** and **5** demonstrates the potential of these compounds as lead structures.

## Experimental Section

**General Experimental Procedures.** <sup>1</sup>H-<sup>13</sup>C HMBC, <sup>1</sup>H-<sup>13</sup>C HMQC, and 2D ROESY spectra were recorded according to the references.<sup>15-18</sup> All other experimental details were as previously reported.<sup>19</sup>

**Bioassays.** Agar diffusion assays were performed as described by Schulz et al.<sup>20</sup> Crude extracts and pure compounds were tested at a concentration of 50 µg/disk. HIV-1-RT inhibition was determined according to the reverse tran-

scriptase assay, nonradioactive (Boehringer Mannheim, cat. no. 1468120), and the work of Eberle and Seibl.<sup>21</sup> Samples were dissolved in 10% DMSO/lysis buffer, resulting in a concentration of 66 µg/mL in the reaction mixture, which contained 1 ng of recombinant HIV-1-RT. Activity toward p56<sup>lck</sup> tyrosine kinase was determined according to the tyrosine kinase assay kit, nonradioactive (Boehringer Mannheim, cat. no. 1534505) with modifications as follows: samples were dissolved in DMSO/H<sub>2</sub>O (1:1), resulting in a final test substance concentration in the reaction mixture of 200 µg/mL, and incubated with 1 µM tyrosine kinase substrate II, 1 mM ATP, 10 mM MgCl<sub>2</sub>, 1 U tyrosine kinase p56<sup>lck</sup> (Upstate Biotechnology), 20% dilution buffer, and 20% assay buffer at 30 °C for 60 to 80 min. Dilution buffer (pH 7.0) contained 25 mM HEPES, 10% glycerol, and 0.1% NP40. Assay buffer (pH 7.5) contained 250 mM Tris, 25 mM NaF, 2.5 mM EDTA-Na<sub>2</sub>, 4.0 mM EGTA, 5 mM DTT, and 150 µM Na<sub>3</sub>VO<sub>4</sub>. Absorbance was measured at 415 nm with an ELISA reader (RAINBOW reader, SLT). Antiviral activity was assayed against diverse hepatitis A virus (HAV) variants. FRhK-4-cells cultivated in 96-well microplates were inoculated with 100 µL of 5 × 10<sup>5</sup> TCID<sub>50</sub>/mL HAV-PI or 2 × 10<sup>7</sup> TCID<sub>50</sub>/ml of a cytopathogenic variant (HAV-cytHB1.1). FRhK-4-cells persistently infected with HAV/7 were also analyzed. Cell morphology was evaluated microscopically within 21 days, and HAV-antigen was detected by immunofluorescence analysis after 21 days. Assays for antiplasmodial activity were carried out using *P. falciparum* K1 (origin of strain: Thailand; resistant to chloroquine and pyrimethamine) and *P. falciparum* NF54 (unknown origin; susceptible to standard antimalarials).<sup>22,23</sup> Cytotoxicity was tested against rat skeletal muscle myoblast (L-6) cells and mouse peritoneal macrophages.<sup>24</sup>

**Animal Material.** The sponge sample was collected by divers using scuba at Nananu-I-Ra, Fiji, from a depth of 15 m. The sponge was identified by Dr. J. N. A. Hooper, Queensland Museum, South Brisbane, Australia. A voucher specimen has been deposited at the Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, Bonn 53115, Germany, voucher no. CT198PPP.

**Extraction and Isolation.** After removal of the preservation EtOH, the sponge tissue was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 0.4 L), followed by MeOH (3 × 0.4 L). The EtOH solution was evaporated to dryness and partitioned between H<sub>2</sub>O/MeOH 50% (0.2 L) and CH<sub>2</sub>Cl<sub>2</sub> (3 × 0.1 L) and combined with the respective extracts to yield 5.2 g of brown gum (MeOH extract) and 1.9 g of brown oil (CH<sub>2</sub>Cl<sub>2</sub> extract). After extraction, the sponge was dried and weighed (80 g). The CH<sub>2</sub>Cl<sub>2</sub> extract was fractionated by normal-phase VLC (gradient elution petrolether/EtOAc/MeOH) to yield 15 fractions each of 80 mL. Four VLC fractions were further purified by normal-phase HPLC; fraction 3 (EtOAc/petrolether 3%) and fraction 4 (EtOAc/petrolether 7%) gave compounds **1** (3 mg) and **2** (3 mg), and fractions 5 and 6 (EtOAc/petrolether 20%) gave isodehydroluffariellolide (**3**) (15 mg). Fractions 11 and 14 were further purified by reversed-phase HPLC (gradient elution MeOH/H<sub>2</sub>O 45:55 to 100:0, 0.05% TFA) to give homofascaplysin A (**4**) (25 mg) and fascaplysin (**5**) (50 mg), respectively.

**Compound 1:** light yellow oil (3 mg, 0.004%); IR (film)  $\nu_{\max}$  2925, 2855, 1755, 1455, 1375, 1345, 1070, 1055 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75.5 MHz), see Table 2; EIMS *m/z* 370 [M<sup>+</sup>] (9), 246 (5), 233 (6), 191 (17), 137 (100); HREIMS *m/z* 370.2860 (calcd for C<sub>25</sub>H<sub>38</sub>O<sub>2</sub>, 370.2872).

**Compound 2:** light yellow oil (3 mg, 0.004%);  $[\alpha]_D^{20}$  ± 0° (*c* 0.0034, CHCl<sub>3</sub>); IR (film)  $\nu_{\max}$  2925, 2865, 1770, 1445, 1375, 1340, 1200, 1135, 1115, 1075, 1020, 940 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100.6 MHz), see Table 2; EIMS *m/z* 414 [M<sup>+</sup>] (7), 369 (1), 290 (1), 272 (2), 137 (100); HREIMS *m/z* 414.3123 (calcd for C<sub>27</sub>H<sub>42</sub>O<sub>3</sub>, 414.3134).

**Isodehydroluffariellolide (3):** colorless oil (13 mg, 0.015%); all other data as previously published.<sup>10</sup>

**Homofascaplysin A (4):** brown oil (25 mg, 0.03%); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  9.35 (1H, d, *J* = 6.6 Hz, H-6), 8.85 (1H, d, *J* = 6.6 Hz, H-7), 8.51 (1H, d, *J* = 8.1 Hz, H-8), 8.29 (1H, d, *J* = 8.1 Hz, H-4), 7.93 (1H, dd, *J* = 1.0, 7.6 Hz, H-1), 7.90 (1H, m, H-11), 7.88 (1H, m, H-10), 7.79 (1H, ddd, *J* = 1.0, 7.6, 8.1

Hz, H-3), 7.72 (1H, ddd,  $J = 1.0, 7.6$  Hz, H-2), 7.56 (1H, ddd,  $J = 1.5, 8.1$  Hz, H-9), 4.29 (1H, d,  $J = 18.3$  Hz, H-14), 4.21 (1H, d,  $J = 18.3$  Hz, H-14), 2.02 (3H, s, H-16);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{-OD}$ , 100.5 MHz)  $\delta$  207.4 (s, C-15), 147.7 (s, C-11a), 145.8 (s, C-12b), 143.5 (s, C-4a), 139.6 (s, C-1a), 137.6 (s, C-7a), 135.0 (d, C-10), 133.1 (d, C-3), 132.8 (d, C-2), 126.7 (d, C-1), 125.6 (d, C-6), 125.3 (d, C-8), 124.6 (d, C-9), 122.3 (s, C-7b), 122.3 (s, C-12a), 119.3 (d, C-7), 116.1 (d, C-4), 115.0 (d, C-11), 80.1 (s, C-13), 52.4 (t, C14), 31.0 (q, C-16); all other data as previously published.<sup>10</sup>

**Fascaplysin (5):** red oil (50 mg, 0.06%);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75.5 MHz), see Table 3; positive FABMS (matrix of glycerol)  $m/z$  271 [ $\text{M}^+$ ], all other data as previously published.<sup>9</sup>

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