Lepadins D−F: Antiplasmodial and Antitrypanosomal Decahydroquinoline Derivatives from the Tropical Marine Tunicate *Didemnum* sp.

Anthony D. Wright,*[,†,‡] Eva Goclik,[†] Gabriele M. König,[†,‡] and Ronald Kaminsky[§]

Institute for Pharmaceutical Biology, Technical University of Braunschweig, Mendelssohnstrasse 1, 38106 Braunschweig, Germany, and Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland

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From a new tunicate species, belonging to the genus *Didemnum*, three alkaloids possessing an unusual and extremely rare decahydroquinoline skeleton and showing significant and selective antiplasmodial and antitrypanosomal activity were obtained as follows: (2*R**,3*S**, 4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methylquinoline (lepadin D, **1**), its quaternary nitrogen derivative (**2**), (2*R**,2″*E*,3*S**,4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl ester 2″-octenoic acid (lepadin E, **3**), and (2*S**,2″*E*, 3*S**, 4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl ester 2″-octenoic acid (lepadin F, **4**). These isolates may well serve as lead structures for the development of new antimalarial drugs.

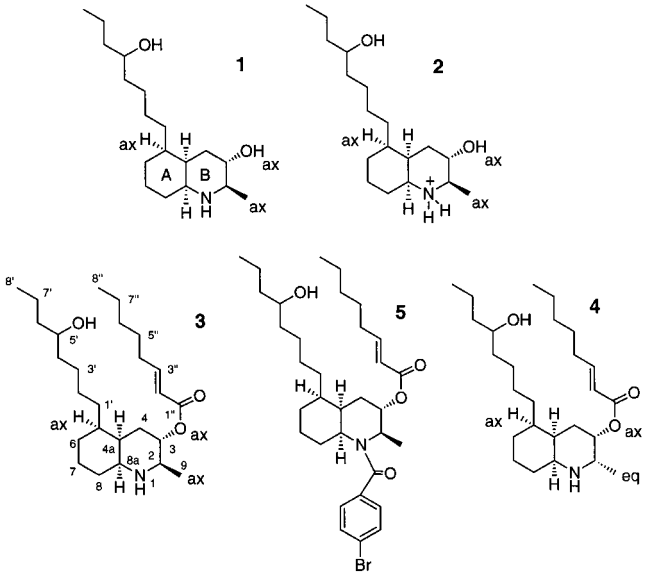
Introduction

The marine environment is still an unexploited resource in terms of antiparasitic agents or lead structures. Some sponge, tunicate, and marine fungal metabolites have, however, clearly demonstrated the potential of marine natural products in this area.[1] Tunicates of the genus *Didemnum* have yielded a variety of secondary metabolites including peptides,[2−4] unusual nine-membered ring lactones,[5] β-carboline,[6,7] and indole-derived alkaloids,[8] lamellarines,[9] didemnenones,[10] enterocin derivatives,[11] pyrroloacridine alkaloids such as plakinidine D,[12] small iodated aromatic compounds,[12,13] and long chain amino alcohols[14] and acids,[15] many of which are halogenated. Several of these compounds were found to have interesting biological activities, e.g., mollamide,[4] cyclodidemnoamide,[2] the didemnenones,[10] and plakinidine D[12] are all cytotoxic against various cancer cell lines, while enterocin[11] and the didemnenones[10] are antimicrobial. In the current project, the antiparasitic activity of *Didemnum*-derived secondary metabolites was investigated. These efforts resulted in the isolation of three new unusual alkaloids (lepadins D−F, **1**, **3**, and **4**) (Chart 1), with antiplasmodial and antitrypanosomal activity, from a new *Didemnum* species collected from Stanley Reef, the Great Barrier Reef. The new compounds are structurally related to the lepadins found in *Clavelina lepadiformis* collected in the North Sea[16,17] and, together with these three compounds,[16,17] are the only decahydroquinoline derivatives known from marine sources.

Results and Discussion

Structural Chemistry. Mass spectrometric (MS) analysis of **1** indicated it to have the molecular formula $C_{18}H_{35}NO_2$. Because there were no multiple bonds present within the molecule, as deduced from the nuclear magnetic resonance (NMR) data, it was evident that the two elements of unsaturation indicated by the molecular formula of **1** must be due to the presence of two rings. From the ^{1}H and ^{13}C NMR data of **1**, it was possible to account for the presence of $C_{18}H_{32}$ (2 × CH_3, 10 × CH_2, and 6 × CH) within the molecule. According to the molecular formula of **1**, this left one nitrogen, two oxygen, and three hydrogen atoms to be accounted for. IR data of **1** showed it to contain OH and/or NH functionality (ν 3290 cm^{-1}). As the ^{13}C NMR data evidenced the presence of two carbons with oxygen (δ 71.2 d, 72.2 d) and two with nitrogen attached (δ 54.0 d, 56.2 d), it was clear that the unaccounted for H_3NO_2 part of **1** was present in the form of two secondary hydroxyl groups and a secondary amine. After all protons had been assigned to their directly bound carbon partner, from the results of a two dimensional (2D) ^{1}H−

Chart 1



* To whom correspondence should be addressed. Tel.: +49 228 73 3747. Fax: +49 228 73 3250. E-mail: a.wright@uni-bonn.de.
† Technical University of Braunschweig.
‡ Current address: Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany. Tel.: +49 228 73 3747. Fax: +49 228 73 3250. E-mail: a.wright@uni-bonn.de.
§ Swiss Tropical Institute.

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Table 1. ^{1}H NMR Data for Compounds **1** (300 MHz, CD_3OD), **2** (400 MHz, CD_3OD), **3** (300 MHz, $CDCl_3$), and **4** (300 MHz, $CDCl_3$)[a]

carbon	1	2	3	4
2	2.99 (dq, J = 4.1, 7.0)	3.46 (dq, J = 2.5, 7.0 Hz)	3.08 m	3.09 (dq, J = 1.9, 6.4 Hz)
3	3.70 (ddd, J = 4.1, 4.3, 4.4 Hz)	3.93 (ddd, J = 2.5, 3.1, 3.1 Hz)	4.78 m	4.92 brs
4	1.50 m, 1.87 (ddd, J = 4.4, 14.0, 14.0 Hz)	1.63 (brd, J = 14.2 Hz), 1.92 (ddd, J = 3.1, 14.2, 14.2 Hz)	1.52 m, 1.89 (ddd, J = 4.5, 11.7, 14.3 Hz)	1.62 (ddd, J = 2.1, 12.3, 12.3 Hz), 1.72 (brd, J = 12.3 Hz)
4a	2.38 m	2.60 m	2.17 m	2.05 m
5	1.53 m	1.59 m	1.47 m	1.42 m
6	1.12 (dddd, J = 3.3, 12.5, 12.5, 12.5 Hz), 1.38 m	1.13 (dddd, J = 3.1, 12.6, 12.7, 12.7 Hz), 1.32 m	1.09 m, 1.38 m	0.93 m, 1.36 m
7	1.79 m	1.89 m	1.13 m, 1.66 m	1.22 m, 1.31 m
8	1.66 (dddd, J = 3.8, 12.2, 12.5, 12.5 Hz), 1.72 m	1.84 m	1.55 m, 1.68 m	1.45 m, 1.78 m
8a	3.03 (ddd, J = 4.9, 6.0, 12.2 Hz)	3.48 m	2.95 m	2.89 (ddd, J = 4.1, 4.1, 12.2 Hz)
9	1.28 (d, J = 7.0 Hz)	1.48 (d, J = 7.1 Hz)	1.19 (d, J = 6.8 Hz)	1.01 (d, J = 6.4 Hz)
1′	1.34 m	1.34 m	1.26 m	1.15 m
2′	1.50 m	1.32 m	1.26 m	1.48 m
3′	1.47 m	1.34 m	1.46 m	1.19 m
4′	1.49 m	1.43 m	1.40 m	1.37 m
5′	3.56 m	3.57 m	3.56 m	3.54 m
6′	1.44 m	1.41 m	1.40 m	1.39 m
7′	1.51 m, 1.40 m	1.46 m	1.39 m	1.38 m
8′	0.97 (t, J = 7.0 Hz)	0.94 (t, J = 6.8 Hz)	0.90 (t, J = 7.0 Hz)	0.9 (t, J = 7.1 Hz)
2″			5.83 (d, J = 15.4 Hz)	5.89 (d, J = 15.4 Hz)
3″			6.99 (ddd, J = 6.8, 6.8, 15.4 Hz)	7.01 (ddd, J = 6.8, 6.8, 15.4 Hz)
4″			2.20 m	2.20 m
5″			1.26 m, 1.40 m	1.20 m
6″			1.29 m	1.31 m
7″			1.31 m	1.30 m
8″			0.90 (t, J = 6.8 Hz)	0.90 (t, J = 7.0 Hz)

[a] All assignments are based on extensive 1D and 2D NMR measurements including COSY, HMQC, and HMBC.

^{13}C heteronuclear multiple quantum coherence (HMQC) experiment, most of the planar structure of the molecule could be deduced from analysis of its ^{1}H−^{1}H correlation spectroscopy (COSY) data. Thus, starting with H_3-9, a continuous chain of ^{1}H−^{1}H coupling, via H-4a to H-5, H-5 to H_2-6, H_2-6 to H_2-7, H_2-7 to H_2-8, and then H_2-8 to H-8a, which further coupled to H-4a, was deduced and enabled ring A within the molecule to be completed. The ^{1}H−^{1}H spin system could be further extended by following the chain of proton couplings from H-5 through to H_3-8′. On the basis of the ^{1}H and ^{13}C NMR chemical shifts of the resonances associated with CH-3 (δ3.70 [ddd, J = 4.1, 4.3, 4.4 Hz] and 71.2 d) and CH-5′ (δ 3.56 m and 72.2 d), it was evident that the two hydroxyl functions must reside at these positions, leaving the NH function to be placed between C-2 and C-8a, and in so doing complete the second ring (ring B) within **1** and its planar structure. The relative stereochemistry of the decahydroquinoline part of the molecule was determined mainly through analysis of ^{1}H−^{1}H coupling constants, and the results of nuclear Overhauser effect (NOE) difference experiments. As all of the ^{1}H−^{1}H coupling constants associated with H-3 were less than 5 Hz, it had to have an equatorial, and the 3-OH an axial, orientation. The coupling constants associated with the axially oriented protons at C-6 (J = 3.3, 12.5, 12.5, and 12.5 Hz) and C-8 (J = 3.8, 12.2, 12.5, and 12.5 Hz) clearly showed H-5 and H-8a (when considered part of the A ring) also to be axially oriented. NOE measurements made with **1** showed interactions to occur between H-4a and H-8a and H-5 and between H-8a and H-5 and H-7_{ax} and revealed H-4a, H-5, and H-8a to be on the same side of the molecule, the A ring to have a chair conformation, and rings A and B to be cis-fused, a deduction also supported by the absence of Bohlman bands in the IR spectrum of **1**.[18] With the conformation of the A ring fixed, the conformation of ring B was also deduced to be a chair on the basis of the fact that the 3-OH group had an axial orientation; H-4 demonstrated a trans-diaxial coupling with H-4a (14.0 Hz) and an axial−equatorial coupling with H-3. The orientation of the methyl group at C-2 was deduced as being axial on the basis of the relatively small ^{1}H−^{1}H coupling constant between H-2 and H-3 (4.1 Hz) and the clear NOE interactions between CH_3-9 and H-4_{ax} and H_2-8. Attempts to resolve the configuration at C-5′ were unsuccessful. For **1**, (2*R**,3*S**,4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methylquinoline, which is similar in structure to lepadins A−C,[16,17] the trivial name of lepadin D, is proposed.

The electron impact (EI) MS data of **2** were almost the same as those for **1**, which indicated that the two compounds were quite similar. Close examination of the ^{1}H and ^{13}C NMR data sets (see Tables 1 and 2) for both compounds supported this deduction and showed the major differences between the two to arise from a change in the B ring. The only alteration in this part of the molecule that was consistent with all of the spectroscopic data was the presence of a quaternary nitrogen between C-2 and C-8a. All of the remaining spectroscopic data for **2**, particularly NOE difference and ROESY measurements, that showed NOE interactions to occur between H-8a and H-4a and H-5 and between CH_3-9 and H-4_{ax} and H_2-8 revealed it to have the same relative configuration as **1** and thus be best described as (2*R**,3*S**,4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methylquinolinium. Clearly, **2** can be generated by the action of dilute acid on **1** (**1** was treated with 0.1 M HCl, and **2** was indeed produced). As the work up of all of the material associated with this

Table 2. ^{13}C NMR Data for Compounds **1** (75.5 MHz, CD_3OD), **2** (100 MHz, CD_3OD), **3** (75.5 MHz, $CDCl_3$), and **4** (75.5 MHz, $CDCl_3$)[a]

carbon	1	2	3	4
2	54.0 d	55.5 d	50.8 d	47.1 d
3	71.2 d	68.0 d	73.4 d	71.3 d
4	24.7 t	21.4 t	22.9 t	23.7 t
4a	33.3 d	31.1 d	33.9 d	33.0 d
5	40.4 d	40.2 d	39.0 d	39.5 d
6	28.3 t	27.3 t	27.5 t	25.8 t
7	25.7 t	25.9 t	23.8 t	26.6 t
8	30.3 t	26.9 t	31.1 t	25.4 t
8a	56.2 d	57.1 d	54.7 d	55.4 d
9	19.6 q	17.4 q	20.7 q	18.4 q
1′	34.4 t	34.0 t	32.9 t	33.1 t
2′	27.0 t	27.6 t	27.3 t	27.7 t
3′	28.3 t	28.0 t	27.7 t	27.0 t
4′	38.4 t	38.3 t	37.5 t	37.5 t
5′	72.2 d[b]	72.1 d	71.6 d	71.6 d
6′	40.8 t	40.8 t	39.8 t	39.8 t
7′	19.9 t	19.9 t	18.9 t	18.9 t
8′	14.5 q	14.5 q	14.0 q	14.2 q
1″			166.0 s	166.6 s
2″			121.4 d	121.2 d
3″			150.0 d	149.9 d
4″			32.2 t	32.2 t
5″			25.8 t	25.2 t
6″			31.4 t	31.4 t
7″			22.5 t	22.5 t
8″			14.2 q	14.0 q

[a] All assignments are based on extensive 1D and 2D NMR measurements including COSY and HMQC. [b] Implied multiplicity by DEPT (C = s, CH = d, CH_2 = t, and CH_3 = q).

tunicate was strictly neutral, however, the likelihood that **2** was an artifact of isolation can be ruled out. It should also be noted that **2** was the only quaternary nitrogen compound detected in this study. As **2** was generated from the action of dilute HCl on **1**, it is not unlikely that the counter ion present in **2** is Cl^-, a deduction that is supported, to some extent, by the chemical ionization (CI) MS of **2**.

Compound **3** was analyzed for $C_{26}H_{47}NO_3$ by HREIMS. Of the four degrees of unsaturation implied by the molecular formula of **3**, two could be attributed to double bonds; a carbon−carbon double bond (δ [5.83 {d, J = 15.4 Hz}, 6.99 {ddd, J = 6.8, 6.8, 15.4 Hz}], 121.4 d, 150.0 d) was detected, which is part of an α,β unsaturated ester carbonyl group (δ166.0 s; 264 nm [ϵ 2730]; 1715 cm^{-1}). As there were no other multiple bonds within the molecule, the two remaining elements of unsaturation had to be present in the form of two rings. From the ^1H and ^{13}C NMR data (see Tables 1 and 2) of **3**, it was possible to deduce that the only difference between it and **1** was the substitution of the secondary hydroxyl function at C-3 in **1** with a 2*E*-octenoic acid ester function. This deduction was supported by all of the spectroscopic data for **3**. The relative configuration of **3** was deduced to be the same as that of **1** based on $^1H-^1H$ coupling constant analysis and NOE difference measurements, which showed it to be (2*R**,2″*E*,3*S**,4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl ester 2″-octenoic acid, for which the trivail name lepadin E is proposed.

MS analysis of **4** showed it to have the identical molecular formula as **3**. All of its other spectroscopic data were also extremely similar to those found for lepadin E (**3**). Interpretation of the HMBC, HMQC, and $^1H-^1H$ COSY spectra of **4** confirmed it to be a decahydroquinoline with the same substitution pattern as that found in **3**; compounds **3** and **4** must thus be stereoisomers. The ^1H and ^{13}C NMR chemical shifts associated with CH-2, CH-3, CH_2-8, and CH_3-9 for **4** differ significantly from those of **3**. When the locations of these atoms within the molecule are considered, it is evident that the only change within the molecule that can be made to accommodate all of these differences, without significantly affecting $^1H-^1H$ coupling constants, is inversion of the configuration at C-2. The results of NOE difference measurements made with **4**, in particular interactions between H-2 and H-4_{ax} and H-8_{ax}, between H-8a and H-4a and H-5, and between CH_3-9 and H-2″ and H-3″, were also consistent with this deduction and showed the molecule to be best described as (2*S**,2″*E*,3*S**,4a*R**,5*R**,8a*S**)-decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl ester 2″-octenoic acid (lepadin F).

Attempts were made to prepare some para-bromobenzoate derivatives of the new lepadins in order to determine the absolute configuration for at least one of the new compounds. In each case, unfortunately, these reactions yielded only amides of the type shown by **5**, which were not crystalline. Esterification at C-5′ was never observed under various reaction conditions.

Compounds **1**−**4** are extremely rare decahydroquinoline derivatives. Among marine natural products, this structural class is only known from the flat worm *Prosthecergeus villatus* and its prey, the tunicate *Clavelina lepadiformis*.[16,17] In the terrestrial environment, poisonous frogs of the genus *Dendrobates* produce simple decahydroquinolines.[19] Among plants, *Lycopdium* species are known to produce decahydroquinolines but as parts of more complex alkaloidal molecules.[20]

Biological Activity. The isolated compounds were tested in several bioassays for their biological activity (Table 3). All compounds, except **2**, had weak antifungal activity against *Ustilago violacea* and/or *Eurotium repens* and several other microorganisms. Compounds **3** and **4** showed moderate inhibition of the enzyme tyrosine kinase $p56^{lck}$ (TK). Antiplasmodial (*Plasmodium falciparum*) and antitrypanosomal (*Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*) activities were assessed for all compounds, and found to be significant and structure-related. Antiplasmodial and antitrypanosomal effects increase significantly from **1** to **4** (e.g., against *P. falciparum* clone K1, **1** has an IC_{50} of 6169 ng/mL [20 μmol] as compared to 208 ng/mL [0.4 μmol] for **4**). This 50-fold activity increase clearly results from the presence of the 2*E*-octenoic acid ester function at C-3 in **3**−**5** in place of the secondary hydroxyl function found in **1** and **2**. Antiplasmodial activity also increases in going from **3** to **4**. As the only difference between these two molecules is the configuration at C-2, it must be concluded that the configuration at this center plays a significant role in determining the activity of the tested molecules. The mechanism of action of these compounds is unknown and needs to be investigated to see if it is similar to that of the fully aromatic quinoline type compounds, e.g., chloroquine.[21] The conformationally mobile side chain at C-3 probably serves to stabilize any nonbonding interactions between the test compounds and a "receptor" molecule, which,

Table 3. Biological Activities of Compounds **1**−**5**

substance tested	*Ustilago violacea*[a]	*Eurotium repens*[a]	tyrosine kinase[b]	*Plasmodium falciparum*, IC_{50} μg/mL strain K1	*Plasmodium falciparum*, IC_{50} μg/mL strain NF54	*Trypanosoma cruzi*, IC_{50} μg/mL	*Trypanosoma rhodesiense*, IC_{50} μg/mL	cytotoxicity[c], IC_{50} μg/mL
chloroquine				0.091	0.005			
qinghaosu				0.001	0.003			
melarsoprol						0.6	0.0026	
miconazol	27	20						
piceatannol			100					
1	2	1	NA	6.1(20)	10.0(33)	37.2	5.60	>30
2	NA[d]	NA	NA	4.0(12)[e]	10.0(33)[e]	37.3	1.97	>30
3	4	4	65	0.4(0.9)	0.9(2)	2.2	0.38	16.2
4	NA	2	86	0.2(0.4)	0.3(0.7)	2.6	0.23	18.3
5				0.5(0.8)	0.4(0.6)		36.85	

[a] Number in this column indicates zone of inhibition, in millimeters, measured from the edge of a circular filter disk impregnated with test substance; sample concentration 0.02 mg/disk. [b] Tyrosine kinase $p56^{lck}$ percent inhibition using 200 μg/mL of sample. [c] Rat skeletal muscle myoblast cells (L6). [d] NA, indicates a substance was inactive in the applied test. [e] On the basis of the counter ion being Cl.

in the case of the quinolines, is heme.[22] Compounds **3** and **4** also have antitrypanosomal effects and are weakly cytotoxic.

Conclusions

Four (**1**−**4**) unusual decahydroquinolines were isolated from a new species of *Didemnum*. The most pronounced biological activities were seen for compounds **3** and **4** in antiplasmodial and antitrypanosomal tests. This biological activity seems to be dependent on the configuration at C-2 and the nature of the functionality at C-3 in the decahydroquinoline. Clearly, the low cytotoxicity of the molecules isolated and tested in this study makes them suitable as models for the development of potential therapeutic agents.

Experimental Section

General Experimental Procedures. These were performed as previously reported.[23]

Animal Material. Animal material was obtained in May 1983, from Stanley Reef, the Great Barrier Reef, Queensland, Australia. Animals growing at 4−7 m depth were collected, deep frozen, and on return to the laboratory, freeze-dried. A voucher specimen is deposited at the Department for Pharmaceutical Biology, Universtiy of Bonn (voucher no. LL48).

Extraction and Isolation. Tunicate tissue (53.0 g) was exhaustively extracted with $(CH_3)_2CO$ (2 L) followed by aqueous MeOH (80%) (2.5 L) to yield 1.76 g of a $(CH_3)_2CO$ soluble material. Normal phase vacuum-liquid chromatography (NP VLC) of the $(CH_3)_2CO$ solubles, using hexane with increasing proportions of EtOAc as eluent, followed by MeOH, afforded 14 fractions each of 110 mL. Thin-layer chromatography (TLC) and ^1H NMR examination of these fractions indicated VLC fraction 12 to be of further interest. VLC separation of this fraction employing normal phase silica and gradient elution from EtOAc to MeOH yielded eight fractions (12.1−12.8) each of 80 mL. VLC separation of fraction 12.2 employing normal phase silica and elution with a 9:1 mixture of CH_2Cl_2 and MeOH afforded a further six fractions (12.2.1−12.2.6). Normal phase column chromatography, employing EtOAc:MeOH:NH_3 8:2:0.1 as eluent, of VLC fraction 12.2.3 yielded compound **2**. Normal phase column chromatography, employing EtOAc:MeOH:NH_3 8:2:0.1 as eluent, of VLC fraction 12.2.4 yielded compound **1**. Normal phase column chromatography, employing EtOAc:$(CH_3)_2CO$:MeOH:H_2O:NH_3 12:43:43:1:1 as eluent, of VLC fraction 12.3 gave a further 11 fractions (12.3.1−12.3.11). Normal phase high-performance liquid chromatography (HPLC) separation of fraction 12.3.3, using EtOAc:MeOH:NH_3 9:1:0.1 as eluent, yielded compound **3**. Normal phase HPLC separation of fraction 12.3.6, using EtOAc:MeOH:NH_3 9:1:0.1 as eluent, yielded compound **4**.

(2*R,3*S**,4a*R**,5*R**,8a*S**)-Decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methylquinoline (Lepadin D, 1).** A clear oil (4 mg, 0.008%); $[\alpha]_D^{22}$ +3.0° (*c* 0.2, MeOH). IR υ_{max} (film): 3290, 3250, 2915, 1460 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2. EIMS: *m*/*z* $[M + H]^+$ 298 (2), $[M]^+$ 297 (6), 282 (4), 264 (10), 254 (54), 236 (28), 168 (100), 119 (36). HREIMS: *m*/*z* 297.266 (calcd for $C_{18}H_{35}NO_2$ $[M]^+$, 297.2668).

(2*R,3*S**,4a*R**,5*R**,8a*S**)-Decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methylquinolinium (2).** A clear oil (9 mg, 0.017%); $[\alpha]_D^{22}$ −14.4° (*c* 0.27, MeOH). IR υ_{max} (film): 3340, 2930, 1455 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2. EIMS: *m*/*z* $[M]^+$ 298 (2), 297 (8), 282 (4), 264 (10), 254 (54), 236 (28), 168 (100). HREIMS: *m*/*z* 297.266 (calcd for $C_{18}H_{35}NO_2$ $[M - H]^+$, 297.2668).

(2*R,2″*E*,3*S**,4a*R**,5*R**,8a*S**)-Decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl Ester 2″-Octenoic Acid (Lepadin E, 3).** A clear oil (15 mg, 0.028%); $[\alpha]_D^{22}$ −2.0° (*c* 0.1, MeOH). IR υ_{max} (film): 3360, 2925, 1715 cm^{-1}. UV λ_{max} (MeOH): 263 nm (e 2110). ^1H and ^{13}C NMR data, see Tables 1 and 2. EIMS: *m*/*z* $[M]^+$ 421 (<1), 420 (<1), 406 (1), 378 (2), 292 (5), 279 (100), 236 (60), 206 (20), 178 (54), 150 (24). HREIMS: *m*/*z* 421.356 (calcd for $C_{26}H_{47}NO_3$, 421.3556).

(2*S,2″*E*,3*S**,4a*R**,5*R**,8a*S**)-Decahydro-3-hydroxy-5-(5′-hydroxyoctyl)-2-methyl-3-quinolinyl Ester 2″-Octenoic Acid (Lepadin F, 4).** A clear oil (10 mg, 0.019%); $[\alpha]_D^{22}$ −1.5° (*c* 0.1, $CHCl_3$). IR υ_{max} (film): 3360, 2925, 1715 cm^{-1}. UV λ_{max} (MeOH): 264 nm (ϵ 2730). ^1H and ^{13}C NMR data, see Tables 1 and 2. EIMS: *m*/*z* $[M]^+$ 421 (<1), 420 (<1), 406 (1), 378 (2), 292 (5), 279 (100), 236 (60), 206 (20), 178 (54), 150 (24). HREIMS: *m*/*z* 421.356 (calcd for $C_{26}H_{47}NO_3$, 421.3556).

To a stirred CH_2Cl_2 solution of **3** (6 mg in 3 mL), 3 mol equiv of para-bromobenzoyl chloride and 2 mg of dimethyl aminopyridine were added. The resultant solution was stirred for 10 h at 24 °C, and the reaction was then quenched with H_2O (5 mL). The H_2O phase was then extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 solubles were purified by normal phase HPLC ($[CH_3]_2CO$:petroleum ether, 50:50 as eluent) to yield **5**.

Para-Bromobenzoyl Derivative of 3 (5). A clear oil (4 mg). ^1H and ^{13}C NMR data, partially assigned data available from the authors by request. EIMS: *m*/*z* $[M]^+$ 605, 603 (4, 4), 562, 560 (4, 5), 463, 461(100, 99). HREIMS: *m*/*z* 603.292 (calcd for $C_{33}H_{50}{}^{79}BrNO_4$, 603.2923).

Biological Testing. The antimicrobial,[24] TK inhibition,[25] reverse transcriptase (RT) inhibition,[26] antimalarial,[27] antitrypanosomal,[28,29] and cytotoxicity[30] assays were carried out as previously described.

Agar Diffusion Test. Test organisms for the agar diffusion assays were the bacteria *Bacillus megaterium* de Bary (Gram positive) and *Escherichia coli* (Migula) Castellani and Chambers (Gram negative), the fungi *Microbotryum violaceum* (Pers.) Roussel, *Eurotium repens* Corda, and the green microalga *Chlorella fusca* Shih Krauss. Samples were prepared by taking 100 μL (200 μg) of the solution of test sample and

pipetting them onto a sterile antibiotic filter disk, which was then placed onto the appropriate growth medium and sprayed with a suspension of the corresponding test organism and subsequently incubated. Zones of inhibition were measured from the edge of filter disks. Samples displaying a growth inhibition zone ≥3 mm and/or a complete inhibition zone ≥1 mm were considered as positive.[24]

Inhibition of TK. The TK inhibition assay was carried out using T cell tyrosine kinase $p56^{lck}$ and a commercial test kit (ELISA). Sample concentrations were 200 μg/mL.[25]

Inhibition of HIV-1 RT. Activity of recombinant HIV-1-RT was measured according to the ELISA protocol established by Eberle and Seibl.[26] Sample concentrations were 66 μg/mL. A sample was considered as active when the RT activity was reduced by 20% or more. All compounds tested in this assay (**1**−**5**) were found to be inactive.

Antimalarial Activity. Cultures of *P. falciparum* (chloroquine-sensitive strain NF54 and chloroquine-resistant strain K1) were maintained in human erythrocytes [(A+, Albumax, (Invitrogen)] in vitro. The antimalarial activity of extracts was assessed using an in vitro radio isotope incorporation method. IC_{50} values of test extracts, reference, and positive control were determined from logarithmic dose−response curves and were expressed as a percentage of inhibited parasite specific ^{3}H-hypoxanthine incorporation.[27] Samples with IC_{50} < 10 μg/mL were considered as active.

Activity Against Trypanosomes of African Origin. Activity of compounds against *T. brucei rhodesiense*, the causative agent of African sleeping sickness, was determined according to Räz et al.[28] Briefly, parasites were propagated axenically in culture medium supplemented with horse serum. Cultures in logarithmic growth phase were propagated for 3 days in the presence of various drug concentrations. Viability of parasites was quantified using the dye Alamar Blue according to Räz et al.[28] Drug sensitivity was regarded as positive when MIC < 100 μg/mL.

Activity of compounds against *T. cruzi* (Tulahuen strain), the causative agent of Chagas disease, was determined according to Buckner et al.[29] Briefly, a *T. cruzi* strain that expresses the *E. coli* β-galactosidase gene was propagated intracellularly in mammalian fibroblast cells in 96 well microtiter plates. Cultures were propagated for 5 days in the presence of drug, before viability of *T. cruzi*, amastigote forms, was determined. Transfected parasites catalyze a colorimetric reaction with chlorophenol red and β-D-galacto-pyranoside as substrate. The reaction was quantified employing an enzyme-linked immunosorbent assay. Drug sensitivity was regarded as positive when MIC < 100 μg/mL.

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References

(1) (a) Gautret, P.; Le Pepe, P.; Biard, J.; Menard, D.; Verbist, J.; Marjolet, M. The effects of bistramides on rodent malaria. *Acta Parasitol.* **1998**, *43*, 50−53. (b) Miyaoka, H.; Shimura, M.; Kimura, H.; Yamada, H. Antimalarial activity of kalihinol A and new related diterpenoids from the okinawan sponge, *Acanthella* sp. *Tetrahedron* **1998**, *54*, 13467−13474. (c) Wright, A. D.; Wang, H.; Gurrath, M.; König, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. Inhibition of heme (FP) detoxification processes underlies the antimalarial activity of terpene isonitrile compounds from marine sponges *J. Med. Chem.* **2001**, *44*, 873−885. (d) König, G. M.; Wright, A. D.; Linden, A. Antiplasmodial and cytotoxic metabolites from the maltese sponge *Agelas oroides*. *Planta Med.* **1998**, *64*, 443−447. (e) Kirsch, G.; König, G. M.; Wright, A. D.; Kaminsky, R. A new bioactive sesterterpene and antiplasmodial alkaloids from the marine sponge *Hyrtios* cf. *erecta*. *J. Nat. Prod.* **2000**, *63*, 825−829. (f) Osterhage, C.; Kaminsky, R.; König, G. M.; Wright, A. D. Ascosalipyrrolidinone A, an antimicrobial alkaloid, from the obligate marine fungus *Ascochyta salicorniae*. *J. Org. Chem.* **2000**, *65*, 6412−6417. (g) D'Ambrosio, M.; Guerriero, A.; Deharo, E.; Debitus, C.; Munoz, V.; Pietra, F. New types of potentially antimalarial agents: epoxy-substituted norditerpene and norsesterpenes from the marine sponge *Diacarnus levii*. *Helv. Chim. Acta.* **1998**, *81*, 1285−1292.

(2) Vazquez, M. J.; Quinoa, E.; Riguera, R.; Ocampo, A.; Iglesias, T.; Debitus, C. Caledonin, a natural peptide bolaphile with Zn^{II} and Cu^{I} complexing properties from the tunicate *Didemnum rodriguesi*. *Tetrahedron Lett.* **1995**, *36*, 8853−8856.

(3) Toske, S. G.; Fenical, W. Cyclodidemnamide: A new cyclic heptapeptide from the marine ascidian *Didemnum molle*. *Tetrahedron Lett.* **1995**, *36*, 8355−8358.

(4) Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. Studies of Australian ascidians. IV Mollamide, a cytotoxic cyclic heptapeptide from the compound ascidian *Didemnum molle*. *Aust. J. Chem.* **1994**, *47*, 61−69.

(5) Lindquist, N.; Fenical, W. Ascidiatrienolides A−C, novel lactonized eicosanoids from the colonial marine ascidian *Didemnum candidum*. *Tetrahedron Lett.* **1989**, *30*, 2735−2738.

(6) Kearns, P. S.; Coll, J. C.; Rideout, J. A. A new β-carboline dimer from an ascidian *Didemnum* sp. *J. Nat. Prod.* **1995**, *58*, 1075−1076.

(7) Schumacher, R. W.; Davidson, B. S. Didemnolides A−D, new N9-substituted β-carbolines from the marine ascidians *Didemnum* sp. *Tetrahedron* **1995**, *51*, 10125−10130.

(8) Fahy, E.; Potts, B. C. M.; Faulkner, D. J.; Smith, K. 6-bromotryptamine derivatives from the gulf of California tunicate *Didemnum candidum*. *J. Nat. Prod.* **1991**, *54*, 564−569.

(9) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. New alkaloids of the lamellarin class from the marine ascidian *Didemnum chartaceum* (Sluiter, 1909). *J. Org. Chem.* **1988**, *53*, 4570−4574.

(10) Lindquist, N.; Fenical, W.; Sesin, D. F.; Ireland, C. M.; Van Duyne, G. D.; Forsyth, C. J.; Clardy, J. Isolation and structure determination of the didemnenones, novel cytotoxic metabolites from tunicates. *J. Am. Chem. Soc.* **1988**, *110*, 1308−1309.

(11) Kang, H.; Jensen, P. R.; Fenical, W. Isolation of microbial antibiotics from a marine ascidian of the genus *Didemnum*. *J. Org. Chem.* **1996**, *61*, 1543−1546.

(12) (a) Smith, C. J.; Venables, D. A.; Hopmann, C.; Salomon, C. E.; Jompa, J.; Tahir, A.; Faulkner, D. J.; Ireland, C. M. Plakinidine D, a new pyrroloacridine alkaloid from two ascidians of the genus *Didemnum*. *J. Nat. Prod.* **1997**, *60*, 1048−1050. (b) Ford, P. W.; Davidson, B. S. Plakinidine D, a new pyrroloacridine alkaloid from the ascidian *Didemnum rubeum*. *J. Nat. Prod.* **1997**, *60*, 1051−1053.

(13) Sesin, D. F.; Ireland, C. M. Iodinated phenethylamine products from a didemnid tunicate. *Tetrahedron Lett.* **1984**, *25*, 403−404.

(14) Searle, P. A.; Molinski, T. F. Structure and absolute configuration of (*R*)-(*E*)-1-aminotridec-5-en-2-ol, an antifungal amino alcohol from the ascidian *Didemnum* sp. *J. Org. Chem.* **1993**, *58*, 7578−7580.

(15) Isaacs, S.; Kashman, Y.; Benayahu, Y. 3-Hydroxy-7-phenyl-4*E*,6*E*-heptadienoic acid from an acsidian *Didemnum granulatum*. *J. Nat. Prod.* **1994**, *57*, 648−649.

(16) Steffan, B. Lepadin A, a decahydroquinoline alkaloid from the tunicate *Clavelina lepadiformis*. *Tetrahedron* **1991**, *42*, 8729−8732.

(17) Kubanek, J.; Williams, D. E.; Dilip de Silva, E.; Allen, T.; Andersen, R. J. Cytotoxic alkaloids from the flatworm *Prostheceraeus villatus* and its tunicate prey *Clavelina lepadiformis*. *Tetrahedron Lett.* **1995**, *36*, 6189−6192.

(18) (a) Tokuyama, T.; Tsujita, T.; Shimada, A.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. Alkaloids from dendrobatid poison frogs: further cis-decahydroquinolines and 8-methylindolizidines. *Tetrahedron* **1991**, *47*, 5401−5414. (b) Wolfe, S.; Schlegel, H. B.; Whangbo, M.-H.; Bernardi, F. On the origin of the Bohlmann bands. *Can. J. Chem.* **1974**, *52*, 3787−3792.

(19) Daly, J. W. Thirty years of Discovering arthropod alkaloids in amphibian skin. *J. Nat. Prod.* **1998**, *61*, 162−172 and references therein.

(20) Tori, M.; Shimoji, T.; Shimura, E.; Takaoka, S.; Nakashima, K., Sono, M.; Ayer, W. A. Four alkaloids, lucidine B, oxolucidine A, lucidine A, and lucidulinone from *Lycopodium lucidulum*. *Phytochemistry* **2000**, *53*, 503−510.

(21) (a) Egan, T. J.; Ross, D. C.; Adams, P. A. Quinoline antimalarial drugs inhibit spontaneous formation of β-haematin (malaria pigment). *FEBS Lett.* **1994**, *352*, 54−57. (b) Chou, A. C.; Fitch, C. D. Control of FP polymerase by chloroquine and other quinoline derivatives. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 422−427. (c) Zhang, J.; Krugliak, M.; Ginsburg, H. The fate of ferriprotoporphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. *Mol. Biochem. Parasitol.* **1999**, *99*, 129−141. (d) Vennerstrom, J. L.; Nuzum, E. O.; Miller, R. E.; Dorn, A.; Gerena, L.; Dande, P. A.; Ellis, W. Y.; Ridley, R. G.; Milhous, W. K. 8-aminoquinolines active against blood stage *Plasmodium falciparum in vitro* inhibit hematin polymerisation. *Antimicrob. Agents Chemother.* **1999**, *43*, 598−602. (e) Wright, A. D.; Wang, H.; König, G. M.; Gurrath, M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. Inhibition of heme (FP) detoxification processes underlies the antimalarial activity of terpene isonitrile compounds from marine sponges *J. Med. Chem.* **2001**, *44*, 873−885 and references therein.

(22) Loria, P.; Miller, S.; Foley, M.; Tilley, L. Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochem. J.* **1999**, *339*, 363−370 and references therein.

(23) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden, A.; Desqueyroux-Faundéz, R. Antimalarial Activity: The search for marine derived natural products which demonstrate selective antimalarial activity. *J. Nat. Prod.* **1996**, *59*, 710−716.

(24) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. Biologically active secondary metabolites of endophytic *Pezicula* species. *Mycol. Res.* **1995**, *99*, 1007−1015.

(25) Wessels, M.; König, G. M.; Wright, A. D. A New Tyrosine Kinase Inhibitor from the Marine Brown Alga *Stypopodium zonale*. *J. Nat. Prod.* **1999**, *62*, 927−930.

(26) Eberle, J.; Seibl, R. A new method for measuring reverse transcriptase activity by ELISA. *J. Virol. Methods* **1992**, *40*, 347−356.

(27) Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciardi, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M.-A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. 4-Aminoquinoline analogues of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846−1854.

(28) Räz, B.; Iten, M.; Grether-Bühler, I.; Kaminsky, R.; Brun, R. A simple fluorometric and colourimetric assay to determine drug sensitivity of african trypanosomes (*T. b. rhodesiens*e and *T. b. gamb*iense) in vitro. *Acta Tropica* **1997**, *68*, 139−147.

(29) Buckner, F. S.; Verlinde, C. L. M. J.; La Flamme, A. C.; Van Voorhis, W. C. Efficient techniques for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing β-galactosidase. *Antimicrob. Agents Chemother.* **1996**, *40*, 2592−2597.

(30) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. The National Cancer Institute: Cancer drug discovery and development program. *Semin. Oncol.* **1992**, *19*, 622−638.

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