Bioactive Natural and Semisynthetic Latrunculins

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Marine-derived macrolides latrunculins A and B, of the Red Sea sponge *Negombata magnifica*, are the first marine natural products that have been found to reversibly bind to actin monomers and to disrupt its organization. Latrunculins are structurally related to many antimicrobial and antiangiogenic macrolides. Several grams of latrunculin B (**1**), together with a new latrunculin named latrunculin T (**2**), were isolated from a recent collection of *N. magnifica*. Semisynthetic modifications of **1**, including acetylation, acetalization, and *N*-hydroxymethylation, afforded four new (**4**, **5**, **7**, **8**) and two known (**6** and **9**) semisynthetic analogues. Specifically, 15-*O*-methyllatrunculin B (**6**) showed a promising antiangiogenic activity in a chick chorioallantoic membrane assay and antimigratory activity in Boyden's chamber assay. Moreover, latrunculin B (**1**) and the new *N*-acetyllatrunculin B (**4**) displayed potent antimigratory activity in a wound-healing assay. Natural and semisynthetic latrunculins showed potent antimicrobial activity against *Candida albicans*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Bacillus cereus*. Latrunculins are potential leads that can be developed as anticancer and antimicrobial agents.

Marine-derived macrolides latrunculins A and B were first reported by Kashman and co-workers from the Red Sea sponge *Negombata magnifica*.[1,2] Latrunculins are able to disrupt microfilament organization, inhibiting microfilament-mediated processes without affecting the organization of microtubular system.[3] They reversibly bind to the cytoskeleton actin monomers, forming a 1:1 complex with G-actin and disrupting its polymerization.[3] Latrunculins are also reported to decrease intraocular pressure and increase outflow facility without corneal effects in monkeys.[4,5] They relax the sphincter and ciliary's muscle with some separation of miotic and accommodative effects, and hence they were patented as possible antiglaucoma leads.[6] Latrunculins are structurally related to the known microtubule disruptors epothilones A and B and the antiangiogenic macrolide rhizoxin.[7,8] Microtubule disruptors are reported recently to show potent antiangiogenic activity at a subtoxic dose.[9] The same report also indicates the possible use of some abandoned highly toxic microtubule-affecting agents as effective antimigratory drugs without being too cytotoxic.[9] This study is reporting the antimigratory, antiangiogenic, and antimicrobial activities of the microfilament disruptors latrunculins and their semisynthetic analogues.

Several semisynthetic analogues of latrunculins A and B were reported in 1989 without any report on their biological activity.[10] The three key pharmacophores responsible for actin binding and bioactivity of the latrunculin structure were defined as the α,β-unsaturated lactone, the free C-15 lactol hydroxy, and thiazolidinone NH moieties.[10] The objectives of the current study were (1) to isolate adequate amounts of major latrunculins and any related minor macrolides; (2) to structurally optimize the major latrunculins using chemical reactions targeting C-15 hydroxy and thiazolidinone NH; and (3) to test the antimigratory, antiangiogenic, and antimicrobial activities of the resulting compounds.

Results and Discussion

Normal-phase flash column chromatography of a chloroform extract of a recent collection of *N. magnifica* followed by repeated Sephadex LH20 column chromatography afforded latrunculin B (**1**) and the new minor compound latrunculin T (**2**). The HRTOF mass spectrum of **2** displayed a molecular ion peak at *m*/*z* 416.1523 [M + Na]$^+$, suggesting the molecular formula $C_{20}H_{27}NO_5S$ and eight degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **2** (Table 1) suggested an acyclic latrunculinic acid with a 4-thiazolin-2-one side chain. The downfield exchangeable proton singlet at δ 9.81 and the ^{13}C quaternary signal at δ 171.0 confirmed the existence of a free carboxylic acid functionality at C-1 in **2**. The ^2J- and ^3J-HMBC correlations of the two methyl groups H_3-19 and H_3-20, along with ^{1}H−^{1}H-COSY data, confirmed the assignment of the C-2−C-10 segment (Figure 1). The oxygenated proton signal at δ 3.83 was assigned to H-11 on the basis of its COSY correlation with H_2-10, which in turn correlated with H_2-9 (Figure 1). The α,β-unsaturated ketone moiety was evident by olefinic proton signals at δ 7.16 (H-13) and 6.66 (H-14) and a ketone carbon at δ_C 178.5 (C-15). H-13 shows ^3J-HMBC correlations with the C-11 and C-15 ketone carbons as well as a ^2J-HMBC correlation to C-14 (Figure 1). Both H-13 and H-11 show COSY correlations to H_2-12. The identity and location of the 4-thizolin-2-one side chain was confirmed through the ^3J-HMBC correlations of the H-17 singlet (δ 7.16) with the α,β-unsaturated ketone carbon C-15 (δ_C 178.5) and the carbonyl carbon C-18 (δ_C 172.5). H-17 also shows a ^2J-HMBC correlation to C-16 (δ_C 135.8).

The stereochemistry of the $\Delta^{2,3}$ and $\Delta^{6,7}$ systems were both assigned as *Z*, similar to those of **1**, on the basis of the carbon chemical shift of the C-19 methyl (δ_C 26.6) and coupling contstants of H-6 and H-7 (10.6 Hz).[11,12] The 8*S*, 11*R* configuration was provisionally suggested for **2**, based on the fact that all latrunculins have the 8*S*, 11*R* configuration or its equivalent.[12] The 8*S*, 11*R* configuration assignment was further supported by the NOESY correlations of both H_3-20 and H-11 to the same proton multiplet at δ 1.52 (H-10a), suggesting their locations on the same side of the molecule (β-oriented). Compound **2** was named latrunculin T, in the manner of the previously reported latrunculins. Latrunculin T is a plausible biosynthetic precursor of latrunculin B by C-1/C-13 lactonization, followed by formation of the C-11/C-15 pyran and C-15 lactol and subsequent reduction of the 4-thiazolin-2-one to latrunculin B's thiazolidin-2-one.

The key pharmacophores for actin-binding activity of latrunculins, and hence for cytotoxicity, were found previously to be the

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Table 1. ^{13}C and ^{1}H NMR Data of Compounds **2**, **4**, and **5**[a]

	2		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	171.0, q-C		165.7, q-C		171.1, q-C	
2	115.1, CH	5.68, brs	117.9, CH	5.70, q (1.5)	115.8, CH	5.68, brs
3	163.8, q-C		155.0, q-C		146.2, q-C	
4	34.1, CH_2	2.85, ddd (14.5, 7.0, 5.5) 2.30, m	35.8, CH_2	2.68, ddd (12.4, 12.4, 4.5) 1.99, m	37.9, CH_2	2.72, m 2.33, m
5	25.7, CH_2	2.24, m 2.11, m	26.9, CH_2	2.37, dddd (13.1, 12.4, 11.4, 4.5), 2.17, m	25.8, CH_2	2.21, m 2.06, m
6	128.6, CH	5.32, ddd (10.6, 9.9, 2.5)	127.6, CH	5.22, ddd (11.4, 11.4, 2.8)	127.5, CH	5.39, ddd (11.5, 10.6, 3.0)
7	138.1, CH	5.14, dd (10.6, 9.9)	135.8, CH	5.02, dd (11.4, 11.0)	136.4, CH	5.10, dd (10.6, 10.2)
8	31.6, CH	2.47, m	28.9, CH	2.44, m	32.8, CH	2.46, m
9	32.0, CH_2	1.31, 2H, m	31.1, CH_2	1.71, m, 109, m	26.7, CH_2	1.55, m 1.42, m
10	33.3, CH_2	1.52, m 1.34, m	31.2, CH_2	1.50, m 1.34, m	33.9, CH_2	1.51, m 1.36, m
11	71.8, CH	3.83, dddd (11.9, 9.8, 5.5, 4.9)	62.5, CH	4.25, dddd (11.0, 11.0, 2.9, 2.8)	72.4, CH	4.94, m
12	40.7, CH_2	2.49, m 2.41, m	35.4, CH_2	1.87, m 1.51, ddd (14.0, 12.8, 2.4)	37.6, CH_2	2.47, m 2.18, m
13	149.3, CH	7.16, ddd (15.4, 8.0, 7.4)	68.7, CH	5.38, m	146.1, CH	7.01, ddd (15.9, 8.0, 7.3)
14	115.9, CH	6.66, d (15.4)	31.9, CH_2	2.05, m 1.99, m	127.8, CH	6.25, d (16.1)
15	178.5, q-C		97.6, q-C		192.7, q-C	
16	135.8, q-C		62.1, CH	3.83, dd (7.7, 7.4)	62.2, CH	3.63, dd (9.0, 6.0)
17	115.9, CH	7.16, s	28.7, CH_2	3.41, 2H, brd (7.4)	31.6, CH_2	3.17, dd (11.9, 9.0) 3.15, dd (11.9, 5.8)
18	172.5, q-C		176.4, q-C		171.8, q-C	
19	26.6, CH_3	1.91, brs	24.2, CH_3	1.88, brs	24.7, CH_3	1.91, brs
20	21.5, CH_3	0.92, 3H, d (6.5)	22.4, CH_3	0.91, 3H, d (6.5)	21.3, CH_3	0.93, 3H, d (6.5)
COOH		9.81, s				
C-15 OH				3.61, s		
N-Ac			176.8, q-C 21.5, CH_3	2.01, 3H, s	170.2, q-C 21.6, CH_3	2.04, 3H, s

[a] In $CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Coupling constants (*J*) are in Hz.

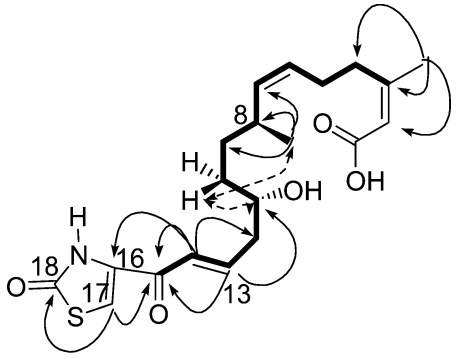


Figure 1. Important HMBC (arrows), COSY (bold), and NOESY (dashed arrows) correlations of **2**.

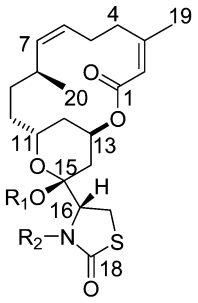
α,β-unsaturated lactone, free C-15 lactol hydroxy, and free thiazolidinone NH group.[2,10,13] Several semisynthetic analogues of latrunculins A and B were reported without any biological activity.[2,10,13] Hence, semisynthetic reactions in this study aimed at preparing both known and new analogues with substituted C-15 hydroxy and NH functionalities and evaluating their biological activities.

Acetylation of **1** using acetic anhydride/pyridine was previously reported. This reaction aimed at opening the lactol and acetylation of the resulting C-11 hydroxy group;[13] however, it did not proceed as expected but resulted in the unexpected 12-membered lactone (**3**) and another linear product.[13] Attempts to acetylate **1** using acetic anhydride with excess Na acetate at 80 °C resulted in two new acetylated analogues, *N*-acetyllatrunculin B (**4**) and compound **5**.

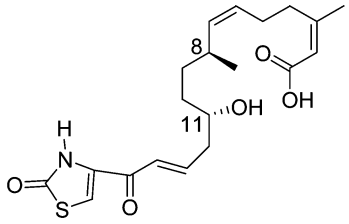
The ESITOF mass spectrum of **4** confirmed the molecular formula of $C_{22}H_{32}NO_6S$ and monoacetylation of latrunculin B. The ^{1}H and ^{13}C NMR data (Table 1) show close similarity to that of **1**, with the replacement of the NH broad singlet in **1** (δ 5.66)[11] by acetate signals (δ_H 2.01, 3H, s; δ_C 21.5, CH_3, and 176.8, q-C). The presence of an exchangeable broad singlet, the C-15-hydroxy, further confirmed *N*-acetylation rather than *O*-acetylation.

The ESITOF mass spectrum of **5** suggested the molecular formula $C_{22}H_{30}NO_5S$. The NMR data of **5** (Table 1) indicated that it is related to the previously reported acetylation product **3** with an *N*-acetylthiazolidin-2-one side chain instead of the 4-thiazolin-2-one in **3**.[13] The downfield shifting of the H-11 multiplet in **5** (δ 4.94) versus that of latrunculin T (δ 3.83) is due to lactone formation at C-11. The identity of the segment C-12 to C-18 was confirmed similar to latrunculin T. The relative stereochemistry of C-8 in **3** was established using spectroscopic and mechanistic (retro-Michael reaction) considerations.[13] The same stereochemistry was assigned for C-8 in **5**, on the basis of the similarity of ^{13}C NMR data of C-8 and C-20 in **3** and **5**. The proton H-11 in **5** was assigned in the α-orientation because it showed NOESY correlation with H-10a (δ 1.51), which in turn showed a correlation with H_3-20. It is assumed that C-16 maintains the original stereochemistry of the parent compound, latrunculin B.

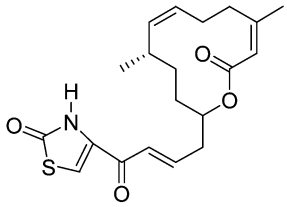
Acetalization of latrunculins with alcohol/ethereal BF_3 was one of the most successful and highest yield (75%) reactions to be reported by Kashman and co-workers in 1989.[10] It was used to produce 15-*O*-alkyl latrunculins with enhanced stability during chemical transformations.[10] Previously reported acetalization analogues of latrunculins were 15-*O*-methyl, ethyl, and propyl acetals. In this study, 15-*O*-methyllatrunculin B (**6**) was prepared using the same reaction as Kashman for the purpose of biological activity testing.[10] To study the effect of extending this side chain, the new derivatives 15-*O*-butyl- (**7**) and 15-*O*-octyllatrunculin B (**8**) were also prepared. Compounds **7** and **8** displayed NMR patterns similar to latrunculin B in addition to new signals for the 15-*O*-butyl and octyl side chains. Hydroxymethylation of **1** afforded the known *N*-hydroxymethyllatrunculin B (**9**).[10] This reaction aimed at evaluating the biological effect of replacing the NH with a primary alcohol.



	R_1	R_2
1 Latrunculin B	H	H
4 *N*-Acetyllatrunculin B	H	CH_3CO
6 15-*O*-Methyllatrunculin B	CH_3	H
7 15-*O*-Butyllatrunculin B	C_4H_9	H
8 15-*O*-Octyllatrunculin B	C_8H_{17}	H
9 *N*-Hydroxymethyllatrunculin B	H	CH_2OH



2 Latrunculin T



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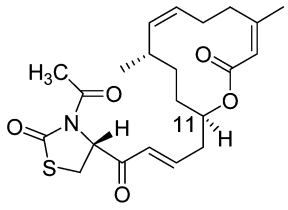

5

Anticancer and Antimicrobial Bioassays. Antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* ATCC 6538 and antifungal activity against *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* were conducted using agar dilution assays.[14,15] Latrunculins **1**, **2**, and **4**−**9** show potent antifungal activity with an MIC range of 2.5−19 μM. This activity is more potent than that of the positive antifungal control, clotrimazole, which displayed an MIC of 21.7 μM. Only compound **5** and 15-*O*-octyllatrunculin (**8**) showed antibacterial activity, with MICs of 17.8 and 14.7 μM, respectively. This activity was better than penicillin G, which was active at 22.4 μM.

The antiangiogenic activity of latrunculins was evaluated using the chick chorioallantoic memberane assay (CAM).[16] 15-*O*-Methyllatrunculin (**6**) showed potent antiangiogenic activity in the CAM assay at a dose of 12 μM/disk. In contrast, the positive antiangiogenic control, curcumin, displayed a comparable activity at a dose of 57 μM/disk. The antiangiogenic activity of the nontoxic 15-*O*-methyllatrunculin is important because latrunculin B did not show an equivalent activity. Hence, the antiangiogenic activity of **6** is due neither to cytotoxicity nor to metabolic transformation to latrunculin B.

15-*O*-Methyllatrunculin B (**6**) and *N*-hydroxymethyllatrunculin B (**9**) inhibited the migration of the murine brain-metastatic melanoma cells (B16B15b) in a Boyden's chamber assay at 1 μM (Figure 2).[17−19] Latrunculin B (**1**) and *N*-acetyllatrunculin B (**4**) inhibited B16B15b tumor cell migration in a wound-healing assay at 1 μM (Figure 3). Latrunculin B (**1**), 15-*O*-butyllatrunculin B (**7**), 15-*O*-octyllatrunculin B (**8**), and the acetylation product **5** inhibited B16B15b tumor cell proliferation using a fluorescence-based cell analysis (Figure 4). These results clearly show the potential of latrunculins, especially the chemically stable nontoxic acetals (**6**−**8**), as possible new lead entities appropriate for further development as anticancer and antimicrobial drugs.

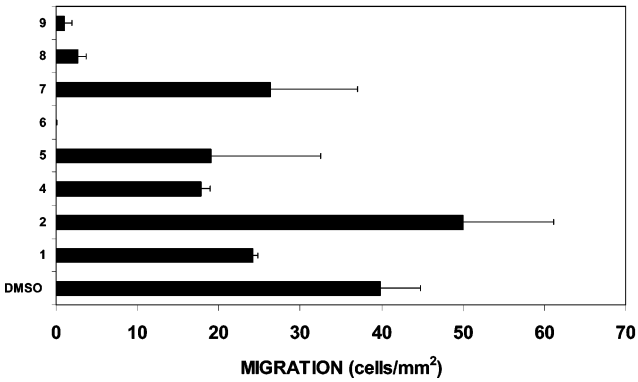

Figure 2. Effects of 1 μM latrunculins **1**, **2**, and **4**−**9** on murine brain-metastatic melanoma cells (B16B15b) migration in a Boyden's chamber assay.

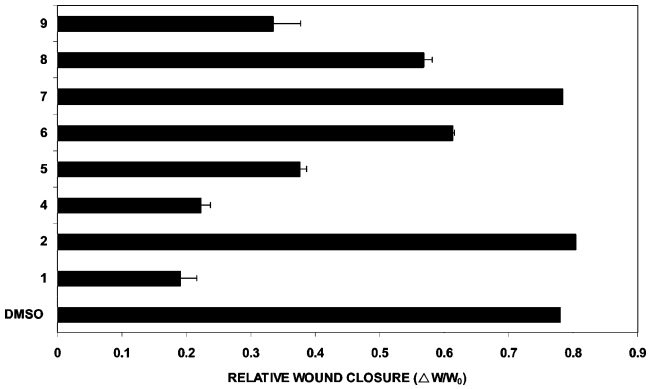

Figure 3. Effects of 1 μM latrunculins **1**, **2**, and **4**−**9** on murine B16B15b tumor cell migration in a wound-healing assay.

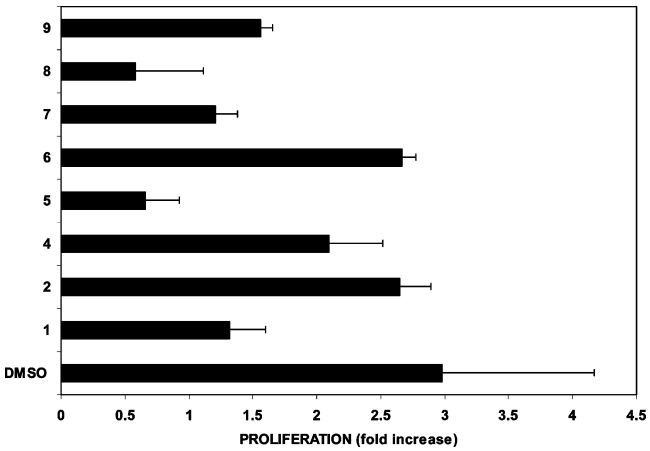

Figure 4. Effects of 1 μM latrunculins **1**, **2**, and **4**−**9** on murine B16B15b tumor cell proliferation using a fluorescence-based cell analysis.

Experimental Section

General Experimental Procedures. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, on a JEOL Eclipse-400 NMR spectrometer operating at 400 MHz for proton and 100 MHz for carbon using TMS

as a standard. The HRESITOF experiments were conducted at the University of Kansas on a Micromass Q-TOF2 spectrometer or at the University of Minnesota on a Bruker BioTOF II spectrometer. TLC analyses were carried out on precoated silica gel G_{254} 500 μm, using the developing systems hexanes−EtOAc (1:1) and $CHCl_3$−MeOH (9:1). For Sephadex LH20 column chromatography, *n*-hexane−$CHCl_3$ (1:1), $CHCl_3$, and $CHCl_3$−MeOH (9:1) systems were used. For column chromatography, Si gel 70−230 mesh was used.

Biological Material. The sponge *Negombata magnifica* Kelly-Borges and Vacelet (order Poecilosclerida, suborder Mycalina, family Podospongiidae) was collected as red long fingerlike strips by scuba from sand bottoms at −10−15 m at Hurghada, on the Egyptian Red Sea coast, in June 2003.[20,21] The sponge was identified by Dr. Rob van Soest, University of Amsterdam. A voucher specimen (03RS3) is deposited in the Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, LA, and in the Red Sea Inverebrates collection at the Department of Pharmacognosy, Suez Canal University, under registration number DY-4.

Extraction and Isolation of Latrunculins. The wet frozen sponge (6 kg) was coarsely minced and percolated with $CHCl_3$ (5 × 1000 mL) at room temperature. The $CHCl_3$ extract was then concentrated under vacuum to give about 250 g of oily extract. This extract was chromatographed over silica gel 60, 40 μm, using a gradient elution system of *n*-hexane−ethyl acetate−MeOH. Latrunculin-containing fractions were repeatedly purified over Sephadex LH20, using a mixture of *n*-hexane−$CHCl_3$ (1:1), to give 3.0 g of latrunculin B. Using a $CHCl_3$−MeOH (9:1) system afforded 21 mg of latrunculin T (**2**), R_f 0.30, silica gel, $CHCl_3$−MeOH (9:1). The identification of **1** was accomplished by comparing its physical and spectral data with reported data.[1,2,11]

Reaction of Ac_2O/Na Acetate with Latrunculin B. A solution of 100 mg of **1** in 2 mL of acetic anhydride was added to 30 mg of anhydrous Na acetate under N_2. The reaction mixture was stirred for 60 min at 80 °C. A brine solution (10 mL) was then added, and the solution was neutralized by $NaHCO_3$ solution. The reaction mixture was extracted with $CHCl_3$ (2 × 15 mL). The organic layer was washed with H_2O (2 × 20 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The reaction residue (90 mg) was fractionated on Si gel 60 (10 g) using $CHCl_3$−MeOH, gradient elution, followed by Sephadex LH20 column chromatography, using a mixture of *n*-hexane−$CHCl_3$ (1:1), to afford **4** (41.0 mg, R_f 0.80, Si gel, $CHCl_3$−MeOH, 9:1) and **5** (16.0 mg, R_f 0.31).

Acetalization of Latrunculin B. Preparation of **6**−**8** was conducted using the previously reported procedure by Blasberger et al.[10] Briefly, a solution of 100 mg of **1** in 1 mL of methanol, butanol, or octanol was treated with 50 μL of ethereal BF_3. Each reaction mixture was stirred for 12 h at room temperature. The solution was then neutralized with 10% aqueous $NaHCO_3$ solution, the alcohol was evaporated under vacuum, and the residue was extracted with $CHCl_3$ (2 × 10 mL). The residue was chromatographed over silica gel using *n*-hexane−EtOAc, followed by Sephadex LH20, to give **6** (76 mg, R_f 0.80, Si gel, *n*-hexane−EtOAc, 1:1), **7** (45 mg, R_f 0.84, Si gel, *n*-hexane−EtOAc, 1:1), and **8** (55 mg, R_f 0.80, Si gel, *n*-hexane−EtOAc, 1:1).

Hydroxymethylation of Latrunculin B. A solution of 100 mg of **1** in 6 mL of ethanol was treated with 3 mL of 35% aqueous CH_2O solution and stirred for 24 h at 60 °C.[10] A brine solution (10 mL) was added, and the mixture was extracted by $CHCl_3$ (2 × 10 mL). The residue was chromatographed on Sephadex LH20 (*n*-hexane−$CHCl_3$ 1:1) to afford **9**.[10]

Antimicrobial Assays. Antibacterial activity against *Bacillus cereus* (isolated from the soft coral *Sarcophyton glaucum* and identified by 16S rDNA) and *Staphylococcus aureus* ATCC 6535 and antifungal activity against *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* (isolated from commercial dry yeast granules) were tested using the agar dilution assay.[14] The MIC values were determined using a modified microtiter plate assay protocol with 24-well format plates, as recommended by the National Committee for Clinical Laboratory Standards.[15]

Chick Embryo Assays. The chick chorioallantoic memberane is an extraembryonic membrane formed on day 4 of incubation by fusion of the chorion and the allantois.[16] Fertilized White Leghorn chicken eggs (Truslow Farms, Chestertown, MD) were placed in an incubator as soon as embryogenesis began and kept under constant humidity at 37 °C. On day 4, the contents of each egg were aseptically transferred to a sterile Petri dish (20 × 100 mm). Dishes were incubated under constant humidity at 37 °C for an additional day. Latrunculins dissolved in ethanol and different concentrations (20.0−1.0 μg/disk) were applied and adsorbed onto inert 1 mm diameter cellulose nitrate disks (VWR). On day 5, these disks were placed on a single blood vessel. Vessel density was quantified by morphometric evaluation of histologic CAM sections fixed at regular intervals after implantation.[16] A no treatment control and a positive control (curcumin with known antiangiogenic activity) were used.[16] Crude curcumin was purchased from Sigma and further purified on silica gel 60 using a mixture of $CHCl_3$−MeOH (9.7:0.3).

Tumor Cell Migration Assays. Migration of murine brain-metastatic melanoma cells (B16B15b) was assayed by means of Transwell cell culture chambers followed by fluorescence plate scanner analysis.[17−19] Cells were added to Transwell upper invasive chambers (12 μm diameter pores) in the presence of 1 or 10 μM of the indicated latrunculins using cell culture medium [DMEM/F12 containing 0.2% bovine serum albumin and penicillin/streptomycin (1%)].[17−19] Cells were allowed to migrate 18 h toward lower chambers using fibronectin (1 μg/mL) and *N*-formyl-met-leu-phe (10 nM) as chemoattractants in DMEM/F12 containing 5% FBS and penicillin/streptomycin. Non-migrating cells were wiped from upper chambers, and migrated cells on the lower filter were stained, mounted, and counted. Results for 1 μM treatments as number of migrated cells/mm^2 are shown in Figure 2. All experiments were performed in triplicate and independently, and means of triplicate determinations and standard deviation values are shown.

Tumor Cell Migration by Wound-Healing Assays. B16B15b cells were plated at 100% confluence and incubated for 18 h to allow for cell attachment.[18] A single wound was made in each well with a 200 μL pipet tip. Disloged cells were then removed by three washes with serum-free medium (SFM), and cells were incubated for 14 h with 1 or 10 μM of the indicated latrunculins in Dulbecco's-modified tissue culture medium (DMEM/F12) containing 5% fetal bovine serum (FBS) and penicillin/streptomycin (1%). Wound width was measured immediately before (W_0) and after 14 h (W_{14}) incubation ($\Delta W = W_0 - W_{14}$) to detect migratory abilities by tumor cells following exposure to each latrunculin. Wound closure values (in mm) are shown in Figure 3 relative to controls (time 0). All experiments were performed in triplicate and independently. Means of triplicate determinations with standard deviation values are shown in Figure 3.

Tumor Cell Proliferation by Fluorescence-Based Cell Analysis. B16B15b cells were plated in growth medium (DMEM/F12 with 5% FBS) in 24-well tissue culture dishes at 2500 cells/well and first incubated at 37 °C to allow for cell attachment.[19] Medium was then replaced with fresh growth medium containing penicillin/streptomycin (1%) and 1 or 10 μM of the indicated latrunculins. Cells were then incubated for 18 h, and medium was replaced with fresh growth medium containing 10% alamarBlue (BioSource International, Camarillo, CA), which is a safe, nontoxic aqueous dye that is used to monitor and assess the innate metabolic activity of cells.[19] Cells were incubated for an additional 3 h, and fluorescence was detected with a Cyto Fluor multi-well plate reader (Cyto Fluor 4000, Applied Biosystems).[19] Cell numbers were calculated using a standard curve. Proliferative values as fold-increase over controls are shown in Figure 4. All experiments were performed in triplicate and independently. Means with standard deviation values are shown in Figure 4.

Latrunculin T (2): colorless oil, $[\alpha]^{25}_D$ +109.5 (*c* 0.11, EtOH); IR ν_{max} (neat) 3688, 3019−2859, 1715−1699, 1680, 1506, 1215, 1100 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESITOF *m*/*z* 416.1523 (M + Na)$^+$ (calcd for $C_{20}H_{27}NO_5SNa$, 416.1508).

***N*-Acetyllatrunculin B (4):** colorless oil, $[\alpha]^{25}_D$ +48.0 (*c* 0.05, EtOH); IR ν_{max} (neat) 3625, 3200, 3019−2840, 1715−1680, 1507, 1240, 1218, 780 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESITOF *m*/*z* 438.1970 (M + H)$^+$ (calcd for $C_{22}H_{32}NO_6S$, 438.1950).

(*R*)-3-Acetyl-4-(*E*)-4-(2*R*,5S,6*Z*,10*Z*)-5,10-dimethyl-12-oxooxacyclododeca-6,10-dien-2-yl)but-2-enoyl)thiazolidin-2-one (5): colorless oil, $[\alpha]^{25}_D$ +113.3 (*c* 0.11, EtOH); IR ν_{max} (neat) 3019−2840, 1720−1680, 1507, 1240, 1215 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESITOF *m*/*z* 420.1865 (M + H)$^+$ (calcd for $C_{22}H_{30}NO_5S$, 420.1845).

15-*O*-Butyllatrunculin B (7): colorless oil, $[\alpha]^{25}_D$ +162.2 (*c* 0.09, EtOH); IR ν_{max} (neat) 3676, 3019−2840, 1710−1680, 1507, 1366, 1213 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.27, m (H_2-1′), 1.46, 2H, m (H_2-2′), 1.32, 2H, m (H_2-3′), and 0.87, 3H, t (6.0), H_3-4′; ^{13}C NMR δ 60.0, CH_2

(C-1′), 31.5, CH_2 (C-2′), 19.6, CH_2 (C-3′), 14.1, CH_3 (C-8′); ESITOF *m*/*z* 474.2283 $(M + Na)^+$ (calcd for $C_{24}H_{37}NO_5SNa$, 474.2290).

15-*O*-Octyllatrunculin B (8): colorless oil, $[\alpha]^{25}_D$ +258.0 (*c* 0.05, EtOH); IR ν_{max} (neat) 3650, 3426, 3017−2859, 1690, 1680, 1453, 1215, 1098 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.28, m (H_2-1′), 1.50−1.06, 12H, m (H_2-2′−H_2-7′), and 0.87, 3H, t (6.5), H_3-8′; ^{13}C NMR δ 60.4, CH_2 (C-1′), 31.9, CH_2 (C-2′), 27.0, CH_2 (C-3′), 29.7, CH_2 (C-4′), 29.4, CH_2 (C-5′), 32.0, CH_2 (C-6′), 21.9, CH_2 (C-7′), 14.2, CH_3 (C-8′); ESITOF *m*/*z* 530.2910 $(M + Na)^+$ (calcd for $C_{20}H_{27}NO_5SNa$, 530.2916).

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Supporting Information Available: ^1H, ^{13}C, MS, COSY, and HMBC NMR spectra of compound **2** are available free of charge via the Internet at http://pubs.acs.org.

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