Published in final edited form as:

ChemMedChem. 2010 February 1; 5(2): 274–285. doi:10.1002/cmdc.200900430.

Semisynthetic Latrunculin Derivatives as Inhibitors of Metastatic Breast Cancer: Biological Evaluations, Preliminary Structure-Activity Relationship, and Molecular Modeling Studies

Mohammad A. Khanfar[†], Daa T. A. Youssef[‡], and Khalid A. El Sayed^{†,*}

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana 71201, Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

Abstract

The microfilament cytoskeleton protein actin plays important role in cell biology and can affect cytokinesis, morphogenesis, and cell migration. These functions usually fail and become abnormal in cancer cells. The marine-derived macrolide latrunculins A and B, from the Red Sea sponge *Negombata magnifica*, are known to reversibly bind actin monomers, forming 1:1 complex with G-actin, disrupting its polymerization. To identify novel therapeutic agents for effective treatment of metastatic breast cancer, several semisynthetic derivatives of latrunculin A (**1**) with diverse steric, electrostatic, hydrogen bond donor and acceptor properties were rationally prepared. Analogues were designed to modulate the binding affinity toward G-actin. Examples of these reactions are esterification, acetylation, and *N*-alkylation. Semisynthetic latrunculins were then tested for their ability to inhibit pyrene-conjugated actin polymerization, and subsequently for their antiproliferative, and anti-invasive properties against MCF7 and MDA-MB-231 cells using MTT and invasion assays, respectively.

Keywords

Actin; Biological evaluation; Invasion; Latrunculin A; Structure-activity relationship

Introduction

Actin is a cytoskeleton protein that forms versatile dynamic polymers, which can define cell polarity, control cell shape and promote stable cell-cell and cell-matrix adhesions.^[1] Actin forms versatile dynamic polymers that can define cell polarity, organize cytoplasmic organelles, control cell shape and promote stable cell-cell and cell-matrix adhesions, and generate protrusive forces required for migration.^[1] These functions usually fail and become abnormal in cancer cells.^[1]

The marine-derived macrolides latrunculins A and B were first reported by Kashman and coworkers from the Red Sea sponge *Negombata magnifica*.^[1,2] Latrunculin A (**1**) was the first marine macrolide known to contain 16-membered ring and the unique 2-thiazolidinone moiety connected via a tetrahydropyran (THP) ring.^[2,3] Latrunculins A and B and derivatives showed antiangiogenic, antiproliferative, antimicrobial, and anti-metastatic activities.^[4,7] The most important biological effect of latrunculins is their ability to disrupt

*To whom Correspondence should be addressed. Telephone 318-342-1725. Fax: 318-342-1737. elsayed@ulm.edu.

[†]University of Louisiana at Monroe.

[‡]Suez Canal University.

microfilament organization, inhibit microfilament-mediated processes without affecting the organization of microtubular system.^[6–14] Latrunculin A reversibly binds the cytoskeleton actin monomers, forming a 1:1 complex with G-actin and disrupting its polymerization.^[6–14] It has striking selectivity, rapid onset of action, and remarkable potency that exceed those of cytochalasin D by 1–2 fold.^[10] Latrunculin A was also reported to decrease intraocular pressure and increase outflow facility without corneal effects in monkeys.^[8,9] Latrunculin A also showed antiviral and antibacterial activities, inhibited stress-activated MAP kinase (SAPK) pathway,^[12] and suppressed hypoxia-induced factor (HIF-1) activation in breast cancer cells.^[5] Therefore, latrunculin A is considered a potential hit appropriate for further optimization studies.

Despite remarkable physiological properties of latrunculins and their widespread use as biochemical tools, the present understanding of the structure-activity relationship (SAR) of these macrolides is fairly limited. Previous attempts of SAR studies were focused on the macrocyclic ring and semi-quantitative testing.^[15,16] X-ray crystallographic structure of latrunculin A bound to actin monomers revealed that both the thiazolidinone and THP moieties are the key pharmacophores that direct the main binding and orientation of latrunculin A inside the ATP-binding cleft.^[13,14] Meanwhile, the macrocyclic ring is involved in hydrophobic interaction and the hydrocarbon chain from C-5 to C-7 is exposed to solvent, forming few contacts with actin and therefore playing a limited binding role.^[13] The detailed binding of **1** to G-actin was identified as follows: C-1 carbonyl oxygen-through water-to glutamate 214 carboxy, C-17 lactol hydroxyl to arginine 210 NH (major binding), C-17 pyran oxygen to tyrosine 69 hydroxy, thiazolidinone NH to aspartate 157 carboxy, and thiazolidinone C-20 carbonyl oxygen to threonine 186 hydroxy.^[13,14] The only hydrogen bond donor (HBD) group was found to be the thiazolidinone NH while the rest of binding functions act as hydrogen bond acceptor (HBA).^[13,14] Therefore, efforts focused on optimization of latrunculins' lactol hydroxyl and thiazolidinone NH should reflect better SAR insights.

Several C-17 lactol hydroxyl and/or thiazolidinone NH-substituted latrunculin A derivatives **2–14** were semisynthesized (Scheme 1 and Table 1). Various aliphatic and aromatic substituents were used. Steric limitation, electrostatic, HBD and HBA properties were varied at these positions and the biological activities of these derivatives were tested. Since actin is essential for cellular proliferation and migration, these activities were quantified by MTT proliferation and cell invasion assays using MCF-7 and MDA-MB-231 breast cancer cell lines.

Results and Discussion

Chemistry

Latrunculin A (**1**) was isolated from the Red Sea marine sponge *Negombata magnifica* and identified by detailed 1D and 2D nuclear magnetic resonance (NMR) studies and a comparison with literature.^[2] Reaction of **1** with methanol or phenethyl alcohol in presence of ethereal BF₃ yielded the corresponding known 17-methoxy (**2**)^[17] and new phenethoxy (**3**) derivatives of **1**, respectively (Scheme 1). The configuration of C-17 was maintained after acetalization of C-17 hydroxyl group. This was confirmed by using molecular modeling and NMR methods. The distance between proton H-18 and the C-17 OCH₃ group in **2** was calculated by SYBYL to be 2.403 Å, which is in the range of NOE coupling. Proton H-18 and C-17 OCH₃ group showed a strong space dipole-dipole coupling in NOESY experiment, confirming a similar β -orientation. The ¹³C chemical shift of carbon C-17 in **2** (δ_C 99.9) was almost identical to the reported value of the same carbon in 17 β -methoxylatrunculin A (δ_C 99.8).^[17] Optical rotation values of **2** and 17 β -

methoxylatrunculin A were almost identical.^[17] Therefore, C-17 β -configuration was maintained in semisynthetic latrunculin A derivatives.

The high resolution mass spectrometry (HR-MS) ¹H, and ¹³C NMR data of **3** indicated the presence of 17-*O*-phenethyl side chain. The downfield shift of the C-17 carbon and H-18 signals in **3** (+3.2 and 0.20 ppm, respectively) compared with that of the starting material **1** suggested possible etherification at C-17.^[2] The doublet of triplet of oxygenated methylene H₂-1' (δ_{H} 3.81, 3.51) showed COSY coupling with the benzylic H₂-2' triplet (δ_{H} 2.89). Protons H₂-1' showed ³JHMBC correlations with C-17 and the aromatic quaternary carbon C-3' (δ_{C} 138.3). Protons H₂-2' showed ³JHMBC correlation with the aromatic methine carbons C-4'/8' (δ_{C} 128.9). Protons H-4'/H-8' showed COSY couplings with protons H-5'/7' and ³JHMBC correlation with C-6' (δ_{C} 126.7).

Compound **4** was prepared by treatment of **1** with benzoic anhydride in CHCl₃ in the presence of 4-dimethylaminopyridine (DMAP) as a catalyst (Scheme 1). Analysis of ¹H, and ¹³C NMR data indicated benzylation at C-17. The HRMS data of **4** suggested the molecular formula C₂₉H₃₅NO₆S. The aromatic double doublet H-3'/7' (δ_{H} 7.69) showed COSY coupling with protons H-4'/6' (δ_{H} 7.42) and ³JHMBC correlation with the carbonyl carbon C-1' (δ_{C} 169.2). Protons H-4'/6' in turn showed COSY coupling with H-5' (δ_{H} 7.53) and ³JHMBC correlation with the aromatic quaternary carbon C-2' (δ_{C} 133.4). The downfield shifting of H-18 (δ_{H} 5.07, >+1.00 ppm) compared with that of the starting material **1**, was possibly due to the anisotropic effect of the newly introduced C-17-*O*-benzoyl functionality.

N-Substitution of **2** was carried as shown in Scheme 1 using NaH in anhydrous THF at 0 °C and the corresponding alkyl and aryl halides to afford **5-11**.^[17] The identity of **5** was confirmed by NMR data analysis which showed the replacement of thiazolidinone NH proton (δ_{H} 5.80) with an *N*-methyl singlet (δ_{H} 2.97 and δ_{C} 36.7) and comparison with literature.^[17] Similarly, the *N*-ethyl functionality in **6** was evident based on the COSY coupling the downfield nitrogenated methylene H₂-1' (δ_{H} 3.71 and 3.35) with the methyl triplet H₃-2' (δ_{H} 1.17). Protons H₂-1' also showed ³JHMBC correlation with the carbonyl C-20, connecting the new ethyl group with the thiazolidinone ring.

The HR-MS data of **7** suggested an additional degree of unsaturation. Analysis of ¹H and ¹³C data further confirmed the new *N*-cyclopentyl moiety. The methine quintet H-1' (δ_{H} 3.88) showed ³JHMBC correlations with the thiazolidinone carbonyl carbon at δ_{C} 171.5 and the methylene carbons C-3'/4' (δ_{C} 24.5). Protons H₂-3'/4' showed COSY coupling with both H₂-2'/5' protons, which in turn COSY-coupled with H-1'.

Analysis of ¹H and ¹³C data of **8** suggested *N*-hydroxypropylation of the starting material **2**. The new methylene singlet H₂-1' (δ_{H} 3.55) showed a ³JHMBC correlation with C-29 carbonyl (δ_{C} 170.2), connecting this moiety to the thiazolidinone ring. Protons H₂-1' also showed a ³JHMBC correlation with the oxygenated methylene carbon C-3' (δ_{C} 59.2). The two chemically unequivalent H₂-3' at (δ_{H} 3.76 and 3.58) showed COSY coupling with H₂-2' (δ_{H} 1.78), confirming the introduction of the new *N*-hydroxypropyl side chain.

The high resolution electrospray ionization mass spectrometry (HRESI-MS) data of **9** suggested the molecular formula C₃₀H₃₉NO₅S, twelve degrees of unsaturation, and the *N*-benzylation of **2**. The downfield benzylic nitrogenate methylene protons H₂-1' (δ_{H} 5.11 and 4.35) showed a ³JHMBC correlation with C-20 carbonyl (δ_{C} 170.2), connecting this moiety to the thiazolidinone ring. Protons H₂-1' also showed a ³JHMBC correlation with the symmetric aromatic methine carbons C-3'/C-7' (δ_{C} 128.4). Protons H-3'/7' showed a ³JHMBC correlation with the aromatic methine carbon C-5' (δ_{C} 127.7) and COSY correlation

with protons H-4'/6' (δ_{H} 7.34). The latter protons also show COSY correlation with H-5' (δ_{H} 7.31) and a $^3\text{JHMBC}$ correlation with the quaternary aromatic carbon C-2' (δ_{C} 132.5).

The ^1H and ^{13}C NMR data of **10** was closely similar to those of **9** with an *N*-benzoyl functionality instead of *N*-benzyl in **9**. The aromatic methine protons H-3'/7' (δ_{H} 7.72) showed a $^3\text{JHMBC}$ correlation with the carbonyl carbon C-1' (δ_{C} 169.7). It is worth noting that benzylation of C-17 lactol hydroxy in **4** and the thiazolidinone NH in **10** resulted in a significant downfield shifting of proton H-18 (+1.22 and +1.49 ppm, respectively) compared with that of **1**, possibly due to the anisotropic effect of the benzene ring and the carbonyl group.

Reaction of **2** with *p*-methoxy phenylacetyl chloride afforded **11**, which was highly unstable in the reaction pot with rapid degradation of the formed amide bond. Therefore; reaction time was shortened for five minutes after reagent addition. The HRESI-MS data of **11** suggested the molecular formula $\text{C}_{32}\text{H}_{41}\text{NO}_7\text{S}$. The ^1H and ^{13}C NMR data of **11** suggested successful *N*-*p*-methoxy phenylacetylation. The methoxy singlet H₃-9' (δ_{H} 3.77) showed a $^3\text{JHMBC}$ correlation with the quaternary aromatic oxygenated carbon C-6' (δ_{C} 159.8). The aromatic protons H-4'/8' (δ_{H} 7.21) showed $^3\text{JHMBC}$ correlations with C-6' and the benzylic methylene C-2' (δ_{C} 42.3). They also show COSY coupling with protons H-5'/7' (δ_{H} 6.81). Proton singlet H₂-2' (δ_{H} 3.79) showed $^2\text{JHMBC}$ correlations with the carbonyl carbon C-1' (δ_{C} 175.7) and the quaternary aromatic carbon C-3' (δ_{C} 126.3). Proton H-18 (δ_{H} 5.30) showed a $^3\text{JHMBC}$ correlation with C-1' carbonyl, connecting the new *p*-methoxy phenylacetyl moiety with the thiazolidinone ring.

To explore the importance of *N*-substitution on the pharmacological effect of unsubstituted C-17 lactol group, compounds **6** and **9** were demethylated by heating with aqueous AcOH to afford **12** and **13**, respectively, with a free C-17 lactol hydroxyl functionality (Scheme 1).^[17] Compounds **12** and **13** showed ^1H and ^{13}C NMR data identical to those of **6** and **9** with the replacement of the C-17 methoxy with a hydroxyl group. The D₂O-exchangeable broad proton singlets at δ 4.01 and 3.91 were assigned as the new C-17 hydroxy signals in **12** and **13**, respectively.

N-Hydroxymethyl latrunculin A (**14**) was prepared as previously reported to study the effect of extending the location of the only HBD in **1** (the thiazolidinone NH) by one carbon and its replacement with a primary alcohol group (Scheme 1).^[17]

Biological Activity

Cytoskeleton is composed of three distinct elements: actin microfilaments, microtubules, and intermediate filaments.^[1,7,10–12] Microtubules form a polarized network, enabling organelle and protein movement throughout the cell.^[7,11] Intermediate filaments are rigid component that maintain the overall cell shape.^[7,11] Actin cytoskeleton and other proteins involved in its regulation and function constitute over 25% of total proteins in the cell.^[1,7,10–12] Cytoskeletal elements coordinately regulate cell's motility, adhesion, migration, exocytosis, endocytosis, and division.^[7,10–12] Actin filaments were found disrupted in the malignant transformed cells.^[1,7,10–12] Alteration of actin polymerization or its remodeling is important in regulating the morphology and phenotypic events of a malignant cell.^[1,7,10–12] Actin alterations are progressive and distinct actin remodeling profiles are correlated with different stages of cancer development and progression.^[1,7,10–12] Compound **1** reversibly binds the cytoskeleton actin monomers, forming a 1:1 complex with G-actin and disrupting its polymerization.^[6–14] Compound **1** showed antiangiogenic, antiproliferative, antimicrobial, and anti-migratory activities and directly correlated with inhibition of diseases where the stability of the cytoskeleton actin is intricately connected to its pathologies.^[1–7,11]

Actin polymerization kit was used to assess the direct actin-binding activity of each analogue at two concentrations; 100 nM and 1 μ M. This kit is based on the enhanced fluorescence of pyrene-conjugated actin that occurs during polymerization. The enhanced fluorescence that results when pyrene-G-actin (monomer) forms pyrene-F-actin can be used to follow polymerization over time.^[18,19] Table 2 lists the activities as percentage of actin polymerization inhibition compared to the negative vehicle (DMSO) control along with calculated IC₅₀. Figure 1 shows the time-course inhibition of actin polymerization for **1** and **3**. Compounds **3** and **14** were profoundly more active than the parent latrunculin A (**1**) in inhibition of actin polymerization at both doses, while compounds **4**, **9**, **12**, and **13** were almost equipotent to **1**. This specific binding assay proves that the antiproliferative and anti-invasive activities of latrunculin A derivatives are due to the disruption of actin polymerization, except compound **11**.

The antiproliferative and anti-invasive activities of **1-14** were quantified using MTT and cell invasion assay kits, respectively. Latrunculin A (**1**) was used as a positive control in all biological assays because it is an established actin polymerization inhibitor and has documented cytotoxic, antiproliferative, and anti-invasive activities.^[1,4-7,10-16] 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay detects the living but not dead cells in quantitative colorimetric fashion utilizing cellular ability to reduce the MTT to insoluble purple formazan dye.^[20,21] Therefore, this method is routinely used to assess the cytotoxicity and proliferation inhibitory activities. The antiproliferative activities of **1-14** were measured against two human mammary gland adenocarcinoma cell lines, MCF7 and MDA-MB-231, at five different doses (0.1, 0.5, 1, 10, and 50 μ M). The calculated IC₅₀ are listed in Table 3. While many of the prepared semisynthetic latrunculins retained the activity, some of them were more active than the starting natural product **1**. Compounds **3** and **14** were more potent than **1** against both cell lines with 4.8 and 4.0-fold increase in the activity against MCF7, respectively, with noticeable cytotoxic effect at doses higher than 1 μ M. Although MDA-MB-231 cells were more resistant than MCF7 against most derivatives, compound **14** retained the potent activity against both cell lines. Even though derivative **10** was less active than **1** in invasion assay, this compound along with **4** and **9** were almost equipotent to the parent natural product **1** in proliferation assay.

Anti-invasive activities of **1-14** were measured using a 96 well basement membrane extract (BME) cell invasion assay kit against the highly metastatic MDA-MB-231 cells.^[22,23] This assay employs a simplified Boyden Chamber design with a polyethylene terephthalate membrane (8 μ). Detection of cell invasion is quantified using calcein- acetomethylester (AM).^[22,23] Cells internalize calcein-AM and intracellular esterases cleave the acetomethylester moiety to generate free calcein. Free calcein fluoresces brightly, and this fluorescence may be used to quantify the number of cells that have invaded across BME.^[22,23] Anti-invasive activities of **1-14** at three different doses are shown in Figure 2. To correlate the anti-invasive activities of latrunculin A derivatives with their actin disrupting effect, **1-14** were tested at non-toxic concentrations. Excluding **3**, MTT assay results showed no or insignificant toxicity at 1.0 μ M for latrunculin derivatives over 72 hours. Therefore, all the compounds were tested at 0.1, 0.5, and 1.0 μ M. Latrunculin **3** killed 17% of MDA-MB-231 cells at 1.0 μ M, while the same dose of **3** inhibited 95% of MDA-MB-231 cells' invasion. This shows a clear difference between the cytotoxic and anti-invasive activity levels. Therefore, the anti-invasive activities for **1-14** are attributed to the inhibition of actin polymerization and not due to cell death.

Derivatives **3**, **11**, **13**, and **14** were 3.3, 4.6, 3.1, and 2.1-fold more active than latrunculin A (**1**), respectively, at 0.5 μ M. 17-*O*-Phenylethyl latrunculin A (**3**) was the most potent, with a 100 nM dose required to inhibit MDA-MB-231 cells' invasion versus 1 μ M dose of **1**. Upon the addition of **1**, **3**, and **14** to MDA-MB-231 cells to the upper assay chamber, rapid

changes in cell morphology were noticed and actin filaments were disrupted characterized by the remarkable cells deformity (data not shown). During cell migration, the cytoskeleton actin is dynamically remodeled, and this reorganization produces the forces necessary for cell migration.^[7,11,12] This suggests that anti-invasive actions of **1**, **3**, and **14** are mediated through direct disruption of actin cytoskeleton remodeling. Interestingly, the second most active derivative, *N*-*p*-methoxyphenylacetyl-15-*O*-methyl-latrunculin A (**11**) showed potent anti-invasive activity at 100 nM dose (Figure 2). This activity was nearly 4-fold more active than **1** at the same dose without antiproliferative, cytotoxicity, or cell shape modifying activities. And because it has weak actin polymerization inhibition (Table 2), compound **11** has different target(s) other than the microfilament actin. While compound **4** and **12** were equipotent to **1** at 1 and 0.5 μ M doses, the rest were less active.

It is reported that latrunculin A binds to actin monomers in the presence of ATP and act by interfering with conformational changes that are necessary for polymerization.^[13,14] Occupation of the latrunculin-binding site clamps ATP in its buried site by preventing the movement between subdomains II and IV.^[13,14] Therefore, it's less likely that latrunculin A or its derivatives can act as ATP-competitive inhibitors.

Preliminary Structure-Activity Relationships

X-ray crystallographic data revealed the binding site of latrunculin A located above the actin nucleotide-binding site in a cleft between subdomains II and IV.^[13,14] The thiazolidinone and THP ring systems were the primary pharmacophores that direct the orientation and binding affinity, while the macrocyclic ring was mainly involved in hydrophobic interaction with no contact with actin at C-5-C-7 chain.^[13,14] Furthermore, the bare macrocyclic system didn't induce polymerization of F-actin at 10 μ M concentration.^[16] Therefore, the two accessible functional groups, C-17 hydroxyl and thiazolidinone nitrogen were utilized to investigate the steric limitations, electronic properties and HBD and HBA characters at these positions in order to probe the chemical tolerance surrounding the binding-determinant amino acids Tyr 69, Asp 157, Thr 186, Glu 214, and Arg 210.

Previous latrunculin derivatives were used as basis to design new derivatives in order to have better understanding of SAR.^[4,5,15,16] Our preceding results revealed the importance of both thiazolidinone and THP ring systems in actin binding.^[4,5] However, clear SAR was not revealed. For example, it has been proved that aliphatic substitution at C-17 lactol hydroxyl decreased the activity,^[4] unlike some aromatic ones, which increased the activity.^[5] *N*-Acetylation of thiazolidinone nitrogen did not disrupt the actin binding although it has strong interaction with Asp 157 and considered the only HBD pharmacophore in the molecule.^[4] Therefore, the chemically accessible C-17 lactol hydroxyl and thiazolidinone NH groups were selected to probe the chemical space around these two critical pharmacophores.

Because actin is involved in numerous normal cellular activities and pathological conditions and due to the high latrunculin A potency and cytotoxicity, this study aims at describing the structural features that can be altered to modulate **1**'s activity. An optimum balance between moderate actin polymerization inhibition and low cytotoxicity can improve the therapeutic index for the use to control cancer invasion and migration, decrease the intraocular pressure in glaucoma, and inhibit tau hyperphosphorylated structures in Alzheimer's disease and other diseases where the stability of the cytoskeleton actin is intricately connected to its pathologies.

Although the internal binding site of latrunculin A is compact, aromatic or aliphatic substitutions with defined steric and electronic properties will not change the binding

orientation or the important interactions as described later by simulated docking for each active derivative.

Despite the fact that C-17 lactol hydroxyl group is acting as a HBA and not HBD, the anti-invasive and antiproliferative activity of methylated derivative (**2**) was extensively suppressed although the methoxy oxygen atom can still act as a HBA (Figure 2, Table 3). This result is consistent with the previously results reported for 15-*O*-methylatrunculin B.⁴ On the other hand, aromatic substitution of thiazolidinone nitrogen recovers the actin-binding activity as in **9** and **10**, but not in **11** where the extra steric effect of the C-9' methoxy group weakens the ligand binding. This outcome can be explained by MOLCAD visualization of docked pose of **9** in the actin-binding site (Figure 3). The benzyl moiety fills the space of Gly 156, Gly 182, and Arg 210 pocket and forms a strong charge-transfer interaction between π aromatic cloud of **9** and the positively-charged guanidine of Arg 210, and possibly Arg 206, while the acetyl moiety in **11** is too long to allow for such interaction with extra steric effect of methoxy group. Because this interaction is absent in *N*-methyl and ethyl substituents of **2**, derivatives **5** and **6** were drastically less active both in the antiproliferation and anti-invasion assays. Surflex Dock software uses Hammerhead screens for the binding of flexible ligand to a protein binding site.^[29,30] Ligand fragments are generated by breaking rotatable bonds and then aligned onto the protomol probes. Highest scoring fragmented poses are retained and proceed until the ligand is complete.^[29,30] This docking procedure allows the *N*-benzyl substitution of **9** to be accommodated between the macrocyclic ring and Arg 210 by rotating the thiazolidinone moiety outward by 2.6 Å and reallocating the macrocycle ring outward by 1.7 Å. Superimposition of docked pose of **9** with the crystallographic structure of **1** (Figure 6) showed that this shift in the thiazolidinone ring position does not disrupt the H-bonding interaction with Thr 186 but rather it places the aromatic ring at an optimum distance near the guanidine moiety of Arg 210 away from the interior compact area (Figures 3 and 6).

Although analogue **11** was dramatically less active than **1** in the antiproliferation assay, it was efficiently potent in attenuating the metastasis of MDA-MB-231. This astonishing observation can be explained by the potential activity of **11** on protein target(s) other than actin as previously noticed with other latrunculin derivatives.^[5,15] It is evident that certain chemical changes in latrunculin A can ultimately change its molecular target.

N-Substitution of **1** with ethyl and benzyl groups preserve the potency of **1** with IC₅₀ 0.63 and 0.95 μ M for **13** and **12**, respectively, against MCF7 whereas MDA-MB-231 was more resistant with IC₅₀ 2.72 for **13** and 7.19 μ M for **12**. This result is consistent with the previously tested *N*-acetylpatrunculin B viewing potent anti-invasive activity against the murine brain-metastatic melanoma cells (B16B15b).^[4] On the other hand, *N*-hydroxypropyl substitution (**8**) reduce the activity to a great extent for two reasons: 1- blocking the C-17 hydroxyl group with no aromatic replacement effect as discussed above and 2- the propyl chain is too long to allow H-bonding interaction between the hydroxyl group and the carboxylate of Asp 157.

The most active derivative in actin binding **3** was docked into the active site of actin using SurFlex Dock to understand the significant potency of **3**. The docked pose (Figure 4) shows optimum electrostatic interaction between the electron cloud of the aromatic ring and the positively-charged guanidine moiety of Arg 210, while maintaining the strong H-bonding interaction with Tyr 69, Asp 157, Thr 186, and Glu 214. The calculated distance using SYBYL 8.0 between the aromatic ring and guanidine moiety was 3.32 Å, which is ideal for electrostatic interaction. This bulky phenylethyl moiety is accommodated between the macrocyclic ring, which is shifted outward and exposed more to the solvent (Figures 4 and 6) and Arg 210 without inappropriate crash or conformational change to actin. In a previous

study, 17-*O*-phenyl-carbamoyl latrunculin A was 5-fold more potent than **1** in invasion assay against PC-3M-CT+ cells.^[5] The calculated length of the phenyl carbamoyl (CONHC₆H₅) moiety from carbonyl carbon to the end of aromatic ring was 6.33 Å, which is very close to that of the phenylethyl moiety of **3** (6.12 Å). Therefore, a distance of 6.10–6.35 Å between aromatic substituents and C-17 hydroxyl group can significantly improve the actin binding affinity of latrunculin A derivatives. It also appears that the C-17 carbamate HBA/HBD feature is not playing a direct role in the ATP binding site of actin.

Latrunculin derivative (**14**) shows improved activity versus the parent latrunculin A (**1**) by 4 and 1.4 folds against MCF7 and MDA-MB-231, respectively, in the proliferation assay, and 2.7 folds against MDA-MB-231 in the invasion assay at 1 µM. This pronounced activity of **14** can be partly explained by improved direct actin binding via the more approachable HBD *N*-hydroxymethylene compared to the thiazolidinone NH in **1** (Figure 5). The *N*-hydroxymethylene proton of **14** can form a strong H-bond with the carboxylate moiety of Asp 157 due to the optimum distance of 2.01 Å between both functionalities (Figure 5) whereas this distance was 2.87 Å in **1** as calculated by SYBYL 8.0. Furthermore, the primary alcohol forms stronger H-bonding than the amide nitrogen of thiazolidinone moiety.

One of the scoring functions that Surflex Dock calculates is crash. Crash is the degree of inappropriate penetration by the ligand into the protein and interpenetration (self-clash) between ligand atoms that are separated by rotatable bonds. Crash scores close to zero are favorable. Negative numbers indicate penetration.^[29] All crash values were in the range of (–) 0.11–0.87, which indicate appropriate penetration with no ligand-protein crash.

Conclusions

Latrunculin A is a potent and selective inhibitor of actin polymerization. To probe the chemical space around the binding-determinant moieties, the C-17 hydroxyl and thiazolidinone nitrogen, they were substituted by various aromatic and aliphatic groups. It has been shown that the C-17/C-15 lactol hydroxyl is essential for actin-binding affinity and therefore noticeably affecting the antiproliferative and anti-invasive activities. Alkyl substitutions on C-17 hydroxyl remarkably diminished biological activity unlike aromatic substitutions at the thiazolidinone nitrogen, which recovers the activity with notable increase in potency. The SAR study of latrunculins provides insights for optimizing their actin activities. An optimum balance between moderate inhibition of actin polymerization and low cytotoxicity can improve the therapeutic index for future use of semisynthetically modified latrunculins to control cancer invasion and migration, decrease the intraocular pressure in glaucoma, and inhibit tau hyperphosphorylated structures in Alzheimer's disease and other diseases where the stability of the cytoskeleton actin is intricately connected to its pathologies.

Experimental Section

General Experimental Procedures

Measurements of optical rotation were carried out on a Rudolph Research Analytical Autopol III polarimeter. IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ¹H and ¹³C NMR spectra were recorded in CDCl₃, using TMS as an internal standard, on a JEOL Eclipse NMR spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C. The HREIMS experiments were conducted at the University of Michigan on a Micromass LCT spectrometer. Analytical HPLC analyses were performed on a DIONEX® Summit II system using Phenomenex Luna 250 × 4.6 mm, RP-C-18 column, and isocratic elution (100% MeOH) with UV detection set at 235 nm to verify the purity of each latrunculin. A purity of >98% has been established for latrunculins **1–14** except analogue **9**,

which showed 91% purity. TLC analysis was carried out on precoated Si gel 60 F₂₅₄ 500 μm TLC plates (EMD Chemicals), using the developing systems *n*-hexane-EtOAc (1:1) or CHCl₃-MeOH (9:1). For CC, Si gel 60 (EMD Chemicals, 63–200 μm), fine Si gel 60 (EM Science, <63 μm), and C-18 Si gel (Bakerbond, Octadecyl 40 μm) were used. For Sephadex LH-20 column chromatography, *n*-hexane-CHCl₃ (1:3), CHCl₃, and CHCl₃-MeOH (9:1) solvent systems were used.

General Chemical Reaction Procedures

(A) Acetalization of C-17 hydroxyl group of latrunculin A: To a solution of **1** in ROH, BF₃-diethyl etherate (Et₂O-BF₃) was added. The mixture was stirred for 12 h at room temperature and was neutralized with 10% aq. NaHCO₃ solution. The solvent was evaporated and the residue extracted with CHCl₃ and dried over MgSO₄. The residue after solvent evaporation was chromatographed over Si gel 60 column.^[17]

(B) Alkylation of the thiazolidinone nitrogen: A solution of **2** in dry THF was gradually added to a suspension of NaH (60% dispersion in mineral oil) in dry THF at 0 °C. The mixture was then stirred for 1 h at 0 °C. Alkyl halide was added and the mixture stirred at rt until **2** completely depleted. Ether and water were then added, the two layers separated, and the organic layer was dried over Na₂SO₄. Evaporation of the solvents afforded a residue, which was chromatographed on Si gel 60 column.^[17]

(C) Demethylation of 17-*O*-methylatrunculin A derivatives: A solution of 17-*O*-methylatrunculin A analogue in AcOH/H₂O/THF (3:1:1) was stirred and heated to 60 oC. The reaction was monitored using TLC till complete depletion of the starting material (~0.5–1 h). The mixture was then cooled to room temperature, neutralized with 10% aqueous NaHCO₃ solution, and ether was then added. The upper organic layer was then washed with water, dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. The residue was chromatographed on a Si gel 60 column.^[17]

17-*O*-Methylatrunculin A (2)—Compound **2** was prepared according to procedure A from **1** (200 mg, 0.72 mmol), MeOH (5 mL), and Et₂O-BF₃ (0.22 mL, 1.77 mmol). Elution with *n*-hexane/EtOAc (8:2) afforded **2** (144 mg, 72.0%).^[17]

17-*O*-Phenylethylatrunculin A (3)—Compound **3** was synthesized according to the general procedure A from **1** (15 mg, 0.036 mmol), phenethanol (1 mL, 7.131 mmol), and Et₂O-BF₃ (15 μL , 0.117 mmol). Elution with *n*-hexane/EtOAc (8:2) afforded **3** (5.5 mg, 36.7%): colorless oil, $[\alpha]_D^{25} + 34.5$ ($c = 0.18$ in CHCl₃); ¹H NMR (400 MHz, CDCl₃): $\delta = 5.41$ (1H, brs, H-2), 2.70 (1H, m, H-4a), 3.57 (1H, dt, $J = 11.9, 3.5$ Hz, H-4b), 2.33 (1H, m, H-5), 5.87 (1H, dt, $J = 15$ and 4.8 Hz, H-6), 6.43 (1H, dd, $J = 15$ and 10.6 Hz, H-7), 6.08 (1H, dd, $J = 10.8$ and 10.6 Hz, H-8), 5.01 (1H, dd, $J = 10.6$ and 10.4 Hz, H-9), 2.81 (1H, m, H-10), 1.11 (1H, m, H-11a), 1.74 (1H, m, H-11b), 1.46 (2H, m, H-12), 4.24 (1H, m, H-13), 1.42 (1H, m, H-14a), 1.66 (1H, m, H-14b), 5.17 (1H, brt, $J = 2.9$ Hz, H-15), 1.89 (1H, dd, $J = 15.4$ and 4.4 Hz, H-16a), 2.15 (1H, m, H-16b), 4.05 (1H, dd, $J = 7.3$ and 7.3 Hz, H-18), 3.07 (1H, dd, $J = 11.4$ and 6.6 Hz, H-19a), 3.13 (1H, dd, $J = 11.4$ and 6.6 Hz, H-19b), 1.91 (3H, s, H₃-21), 0.98 (3H, d, $J = 6.2$ Hz, H₃-22), 3.51 (1H, dt, $J = 8.8$ and 7.0 Hz, H-1'a), 3.81 (1H, dt, $J = 8.8$ and 7.0 Hz, H-1'b), 2.89 (2H, t, $J = 7.3$ Hz, H-2'), 7.17 (1H, dd, $J = 8.4$ and 1.8 Hz, H-4'), 7.29 (1H, m, H-5'), 7.22 (1H, brd, $J = 7.0$ Hz, H-6'), 7.29 (1H, m, H-7'), 7.17 (1H, dd, $J = 8.4$ and 1.8 Hz, H-8'), 5.68 (1H, s, NH); ¹³C NMR (75 MHz, CDCl₃): $\delta = 166.4$ (qC, C-1), 118.2 (CH, C-2), 158.1 (qC, C-3), 30.7 (CH₂, C-4), 31.6 (CH₂, C-5), 132.3 (CH, C-6), 124.3 (CH, C-7), 127.4 (CH, C-8), 135.8 (CH, C-9), 29.8 (CH, C-10), 32.2 (CH₂, C-11), 31.2 (CH₂, C-12), 63.5 (CH, C-13), 35.3 (CH₂, C-14), 67.0 (CH, C-15), 29.8 (CH₂, C-16), 100.1 (qC, C-17), 57.1 (CH, C-18), 27.9 (CH₂, C-19), 175.2 (qC, C-20), 21.8 (CH₃,

C-21), 25.3 (CH₃, C-22), 61.6 (CH₂, C-1'), 36.9 (CH₂, C-2'), 138.3 (qC, C-3'), 128.9 (CH, C-4'), 128.6 (CH, C-5'), 126.7 (CH, C-6'), 128.6 (CH, C-7'), 128.9 (CH, 8') ppm; IR (neat): $\nu_{\max}$ = 3528, 3416, 2927, 2855, 1687, 1455, 1132, 1090, 1020, 1984 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₃₀H₃₉NO₅SNa: 548.2447, found: 548.2463.

17-O-Benzoyllatrunculin A (4)—A solution of **1** (20 mg, 0.047) in anhydrous CHCl₃ (3 mL) was stirred with benzoic anhydride (11 mg, 0.050 mmol) and catalytic amount of DMAP for 12 h at rt. The reaction mixture was neutralized with NaHCO₃ solution. The organic layer was washed with H₂O (2 × 5 mL), dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. The reaction residue was chromatographed over Si gel 60 using isocratic *n*-hexane/EtOAc (9:1) to afford **4** (4.7 mg, 23.5%): colorless oil, $[\alpha]_D^{25}$ +47.1 (c = 0.25 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.62 (1H, brs, H-2), 2.36 (1H, dt, J = 13.2 and 7.3 Hz, H-4a), 3.02 (1H, dt, J = 13.2 and 7.32 Hz, H-4b), 2.22 (2H, dt, J = 10.8 and 6.2 Hz, H-5), 5.67 (1H, dt, J = 15.4 and 6.2 Hz, H-6), 6.20 (1H, dd, J = 15.4 and 10.6 Hz, H-7), 5.94 (1H, dd, J = 11.0 and 11.0 Hz, H-8), 4.89 (1H, dd, J = 10.6 and 10.6 Hz, H-9), 2.50 (1H, m, H-10), 1.01 (1H, m, H-11a), 1.70 (1H, m, H-11b), 1.48 (2H, m, H-12), 3.92 (1H, m, H-13), 1.01 (1H, m, H-14a), 1.69 (1H, m, H-14b), 5.19 (1H, t, J = 4.0 Hz, H-15), 1.63 (1H, m, H-16a), 1.81 (1H, m, H-16b), 5.07 (1H, dd, J = 7.0 and 7.0 Hz, H-18), 3.43 (1H, dd, J = 11.0 and 6.6 Hz, H-19a), 3.53 (1H, dd, J = 11.0 and 6.6 Hz, H-19b), 1.88 (3H, s, H₃-21), 0.66 (3H, d, J = 6.2 Hz, H₃-22), 7.69 (1H, dd, J = 8.0 and 1.5 Hz, H-3'), 7.42 (1H, dd, J = 8.0 and 7.4 Hz, H-4'), 7.53 (1H, dd, J = 7.4 and 1.5 Hz, H-5'), 7.42 (1H, dd, J = 8.0 and 7.4 Hz, H-6'), 7.69 (1H, dd, J = 8.0 and 1.5 Hz, H-7'), 5.61 (1H, s, NH); ¹³C NMR (75 MHz, CDCl₃): δ = 166.6 (qC, C-1), 118.3 (CH, C-2), 157.1 (qC, C-3), 33.2 (CH₂, C-4), 30.7 (CH₂, C-5), 132.5 (CH, C-6), 126.2 (CH, C-7), 127.8 (CH, C-8), 135.9 (CH, C-9), 29.3 (CH, C-10), 31.5 (CH₂, C-11), 30.9 (CH₂, C-12), 64.3 (CH, C-13), 35.0 (CH₂, C-14), 71.0 (CH, C-15), 35.7 (CH₂, C-16), 97.7 (qC, C-17), 61.2 (CH, C-18), 29.7 (CH₂, C-19), 172.1 (qC, C-20), 24.8 (CH₃, C-21), 21.5 (CH₃, C-22), 169.2 (qC, C-1'), 133.4 (qC, C-2'), 129.4 (CH, C-3'), 128.2 (CH, C-4'), 133.1 (CH, 5'), 128.2 (CH, C-6'), 129.4 (CH, C-7') ppm; IR (neat): $\nu_{\max}$ = 3694, 3522, 3202, 2928, 2956, 1690, 1602, 1451, 1378, 1281, 1153, 1118, 971 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₂₉H₃₅NO₆SNa: 548.2083, found: 548.2087.

17-O-Methyl-N-methylatrunculin A (5)—Compound **5** was prepared according to procedure B using **2** as a starting material (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and MeI (0.1 mL, 1.600 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **5** (7.1 mg, 70.1%).^[17]

17-O-Methyl-N-ethylatrunculin A (6)—Compound **6** was prepared according to procedure B using **2** as a starting material (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and EtI (0.1 mL, 1.147 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **6** (7.6 mg, 70.6%): colorless oil, $[\alpha]_D^{25}$ +33.7; (c = 1.3 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.63 (1H, brs, H-2), 2.61 (1H, m, H-4a), 2.89 (1H, m, H-4b), 2.25 (2H, m, H-5), 5.78 (1H, dt, J = 15.4 and 4.0 Hz, H-6), 6.37 (1H, dd, J = 15.4 and 11.0 Hz, H-7), 6.04 (1H, dd, J = 10.9 and 10.6 Hz, H-8), 4.99 (1H, dd, J = 10.6 and 10.6 Hz, H-9), 2.84 (1H, m, H-10), 1.11 (1H, m, H-11a), 1.76 (1H, m, H-11b), 1.46 (2H, m, H-12), 4.16 (1H, m, H-13), 1.07 (1H, m, H-14a), 1.72 (1H, m, H-14b), 5.14 (1H, brt, J = 4.0 Hz, H-15), 1.79 (dd, J = 15.4 and 4.4 Hz, H-16a), 2.13 (1H, m, H-16b), 4.03 (1H, dd, J = 9.9 and 3.0 Hz, H-18), 3.25 (1H, dd, J = 11.7 and 3.0 Hz, H-19a), 3.35 (1H, m, H-19b), 1.91 (3H, brs, H₃-21), 1.02 (3H, d, J = 6.24 Hz, H₃-22), 3.35 (1H, dq, J = 17.4, 7.0 Hz, H-1'a), 3.71 (1H, dq, J = 17.4 and 7.0 Hz, H-1'b), 1.17 (3H, t, J = 7.0 Hz, H-2'), 3.33 (3H, s, O-CH₃); ¹³C NMR (75 MHz, CDCl₃): δ = 167.3 (qC, C-1), 118.5 (CH, C-2), 158.5 (qC, C-3), 32.5 (CH₂, C-4), 30.7 (CH₂, C-5), 132.3 (CH, C-6), 125.0 (CH, C-7), 127.8 (CH, C-8), 135.6 (CH,

C-9), 29.4 (CH, C-10), 31.4 (CH₂, C-11), 31.3 (CH₂, C-12), 63.2 (CH, C-13), 35.1 (CH₂, C-14), 66.7 (CH, C-15), 35.2 (CH₂, C-16), 101.2 (qC, C-17), 60.6 (CH, C-18), 25.5 (CH₂, C-19), 173.6 (qC, C-20), 25.1 (CH₃, C-21), 21.8 (CH₃, C-22), 40.4 (CH₂, C-1'), 29.7 (CH₃, C-2'), 47.6 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\max}$ = 3693, 3601, 2957, 2924, 1681, 1604, 1282, 1163 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₂₅H₃₇NO₅Na, 486.2290, found: 486.2281.

17-O-Methyl-N-cyclopentanelatrunculin A (7)—Compound **7** was prepared according to procedure B starting with **2** (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and iodocyclopentane (0.1 mL, 0.867 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **7** (7.6 mg, 70.6%): colorless oil, $[\alpha]_D^{25} +53.6$ (c = 0.63 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.64 (1H, brs, H-2), 1.41 (2H, m, H-4), 2.28 (2H, m, H-5), 5.80 (1H, dt, J = 15.4 and 3.6 Hz, H-6), 6.37 (1H, dd, J = 15.4 and 11.0 Hz, H-7), 6.04 (1H, dd, J = 11.0 and 10.6 Hz, H-8), 4.99 (1H, dd, J = 10.6 and 10.6 Hz, H-9), 2.83 (1H, m, H-10), 1.13 (1H, m, H-11a), 1.50 (1H, m, H-11b), 0.91 (1H, m, H-12a), 1.61 (1H, m, H-12b), 4.16 (1H, m, H-13), 1.41 (1H, m, H-14a), 1.61 (1H, m, H-14b), 5.16 (1H, brt, J = 3.0 Hz, H-15), 1.75 (1H, m, H-16a), 1.86 (1H, m, H-16b), 3.96 (1H, dd, J = 9.5 Hz, H-18), 3.42 (1H, m, H-19a), 3.47 (1H, dd, J = 9.9 and 6.6 Hz, H-19b), 1.91 (3H, s, H₃-21), 1.02 (1H, d, J = 6.2 Hz, H₃-22), 3.88 (1H, t, J = 8.8 Hz, H-1'), 2.14 (2H, m, H-2'), 1.81 (2H, m, H-3'), 2.05 (2H, m, H-4'), 1.81 (2H, m, H-5'), 3.28 (3H, s, O-CH₃); ¹³C NMR (75 MHz, CDCl₃): δ = 166.5 (qC, C-1), 118.5 (CH, C-2), 157.7 (qC, C-3), 31.3 (CH₂, C-4), 28.6 (CH₂, C-5), 132.4 (CH, C-6), 125.0 (CH, C-7), 127.8 (CH, C-8), 135.6 (CH, C-9), 29.8 (CH, C-10), 30.8 (CH₂, C-11), 29.5 (CH₂, C-12), 63.9 (CH, C-13), 35.0 (CH₂, C-14), 66.7 (CH, C-15), 32.4 (CH₂, C-16), 101.6 (qC, C-17), 63.1 (CH, C-18), 26.2 (CH₂, C-19), 171.7 (qC, C-20), 25.1 (CH₃, C-21), 21.8 (CH₃, C-22), 60.7 (CH, C-1'), 29.9 (CH₂, C-2'), 24.5 (CH₂, C-3'), 24.5 (CH₂, C-4'), 29.9 (CH₂, C-5'), 47.5 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\max}$ = 3696, 3672, 2928, 2855, 1685, 1602, 1290 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₂₅H₃₇NO₅Na, 526.2603, found: 526.2603.

17-O-Methyl-N-(3'-hydroxypropyl)atrunculin A (8)—Compound **8** was prepared according to procedure B from **2** (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and 3-iodo-1-propanol (0.1 mL, 1.043 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **8** (4.6 mg, 40.6%): colorless oil, $[\alpha]_D^{25} +53.6$ (c = 0.83 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.64 (1H, s, H-2), 2.24 (2H, m, H-4), 2.25 (2H, m, H-5), 5.79 (1H, dt, J = 5.4 and 5.5 Hz, H-6), 6.39 (1H, dd, J = 5.0 and 11.0 Hz, H-7), 6.04 (1H, dd, J = 10.7 and 10.6 Hz, H-8), 5.01 (1H, dd, J = 10.6 and 10.6 Hz, H-9), 2.84 (1H, m, H-10), 1.09 (1H, m, H-11a), 1.65 (1H, m, H-11b), 1.43 (2H, m, H-12), 4.17 (1H, m, H-13), 1.46 (1H, m, H-14a), 1.77 (1H, m, H-14b), 5.15 (1H, brt, J = 3.0 Hz, H-15), 1.81 (1H, m, H-16a), 2.01 (1H, m, H-16b), 4.01 (1H, dd, J = 9.9 and 4.0 Hz, H-18), 3.27 (1H, dd, J = 12.8 and 4.5 Hz, H-19a), 3.37 (1H, m, H-19b), 1.91 (3H, s, H₃-21), 1.01 (3H, d, J = 6.6 Hz, H₃-22), 3.55 (2H, m, H-1'), 1.78 (2H, m, H-2'), 3.58 (1H, m, H-3'a), 3.76 (1H, dt, J = 8.1 and 5.8 Hz, H-3'b), 3.31 (3H, s, O-CH₃), 5.64 (s, OH); ¹³C NMR (75 MHz, CDCl₃): δ = 166.3 (qC, C-1), 118.3 (CH, C-2), 154.2 (qC, C-3), 30.2 (CH₂, C-4), 29.8 (CH₂, C-5), 132.3 (CH, C-6), 124.8 (CH, C-7), 127.9 (CH, C-8), 153.6 (CH, C-9), 29.7 (CH, C-10), 31.4 (CH₂, C-11), 31.2 (CH₂, C-12), 63.3 (CH, C-13), 35.2 (CH₂, C-14), 66.5 (CH, C-15), 32.4 (CH₂, C-16), 101.0 (qC, C-17), 61.4 (CH, C-18), 29.4 (CH₂, C-19), 170.2 (qC, C-20), 25.2 (CH₃, C-21), 21.8 (CH₃, C-22), 41.8 (CH₂, C-1'), 30.8 (CH₂, C-2'), 59.2 (CH₂, C-3'), 47.5 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\max}$ = 3694, 3601, 3505, 2929, 2857, 2360, 1671, 1602, 1456, 1291, 1127, 1089 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₂₅H₃₇NO₅Na, 516.2396, found: 516.2388.

17-O-Methyl-N-benzylatrunculin A (9)—Compound **9** was prepared according to procedure B using **2** as a starting substrate (15 mg, 0.033 mmol), in THF (1 mL), NaH (2

mg, 0.041 mmol) in THF (1 mL) and benzyl chloride (0.1 mL, 0.866 mmol), elution with *n*-hexane/EtOAc (9:1) afforded **9** (11.3 mg, 75.3%): colorless oil, $[\alpha]_D^{25} +28.5$ ($c = 0.48$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.63$ (1H, brs, H-2), 1.91 (1H, m, H-4a), 3.45 (1H, dt, $J = 13.6$ and 11.0 Hz, H-4b), 2.26 (2H, m, H-5), 5.82 (1H, dt, $J = 13.0$ and 5.1 Hz, H-6), 6.35 (1H, dd, $J = 15.4$ and 11.0 Hz, H-7), 6.04 (1H, dd, $J = 10.8$ and 10.6 Hz, H-8), 5.01 (1H, dd, $J = 10.6$ and 11.4 Hz, H-9), 2.80 (1H, m, H-10), 0.89 (1H, m, H-11a), 1.67 (1H, m, H-11b), 1.42 (1H, m, H-12a), 1.61 (1H, m, H-12b), 4.19 (1H, m, H-13), 1.38 (1H, m, H-14a), 1.88 (1H, m, H-14b), 5.15 (1H, brt, $J = 3.0$ Hz, H-15), 1.51 (1H, m, H-16a), 1.95 (1H, m, H-16b), 3.82 (1H, dd, $J = 9.2$ and 2.9 Hz, H-18), 3.23 (1H, dd, $J = 15.0$ and 2.6 Hz, H-19a), 3.28 (1H, dd, $J = 15.0$ and 3.0 Hz, H-19b), 1.90 (3H, s, H₃-21), 1.01 (3H, d, $J = 6.2$ Hz, H₃-22), 4.35 (1H, d, $J = 14.6$ Hz, H-1'a), 5.11 (1H, d, $J = 14.6$ Hz, H-1'b), 7.23 (1H, dd, $J = 8.4$ and 1.4 Hz, H-3'), 7.34 (1H, dd, $J = 7.3$ and 1.4 Hz, H-4'), 7.31 (1H, dd, $J = 8.4$ and 7.3 Hz, H-5'), 7.34 (1H, dd, $J = 7.3$ and 1.4 Hz, H-6'), 7.23 (1H, dd, $J = 8.4$ and 1.4 Hz, H-7'), 3.17 (3H, s, O-CH₃); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 166.5$ (qC, C-1), 118.4 (CH, C-2), 157.8 (qC, C-3), 31.4 (CH₂, C-4), 29.8 (CH₂, C-5), 132.4 (CH, C-6), 124.9 (CH, C-7), 127.9 (CH, C-8), 135.6 (CH, C-9), 29.7 (CH, C-10), 30.7 (CH₂, C-11), 30.4 (CH₂, C-12), 63.4 (CH, C-13), 35.2 (CH₂, C-14), 66.9 (CH, C-15), 32.5 (CH₂, C-16), 101.7 (qC, C-17), 59.7 (CH, C-18), 25.5 (CH₂, C-19), 173.4 (qC, C-20), 25.2 (CH₃, C-21), 21.8 (CH₃, C-22), 48.3 (CH₂, C-1'), 132.5 (qC, C-2'), 128.4 (CH, C-3'), 128.8 (CH, C-4'), 127.7 (CH, C-5'), 128.8 (CH, C-6'), 128.4 (CH, C-7'), 47.6 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\text{max}} = 3523$, 3371, 2897-2816, 1731, 1744, 1544, 1344, 1354 cm^{-1} ; HRMS-ESI: m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{25}\text{H}_{37}\text{NO}_5\text{SNa}$, 548.2447, found: 548.2443.

17-O-Methyl-N-benzoyllatrunculin A (10)—Compound **10** was prepared according to procedure B using **2** as a starting substrate (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and benzoyl chloride (0.1 mL, 0.866 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **10** (5.2 mg, 52.0%): colorless oil, $[\alpha]_D^{25} +42.7$ ($c = 0.15$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.65$ (1H, brs, H-2), 2.26 (1H, dt, $J = 13.2$ and 7.3 Hz, H-4a), 3.48 (1H, dt, $J = 13.3$ and 11.0 Hz, H-4b), 2.25 (2H, m, H-5), 5.76 (1H, dt, $J = 14.6$ and 6.2 Hz, H-6), 6.34 (1H, dd, $J = 14.6$ and 11.7 Hz, H-7), 5.99 (1H, dd, $J = 11.0$ and 10.8 Hz, H-8), 4.89 (1H, dd, $J = 11.0$ and 10.8 Hz, H-9), 2.71 (1H, m, H-10), 0.77 (1H, m, H-11a), 1.13 (1H, m, H-11b), 1.60 (2H, m, H-12), 4.05 (2H, m, H-13), 1.33 (1H, m, H-14a), 1.63 (1H, m, H-14b), 5.25 (1H, brt, $J = 3.3$ Hz, H-15), 2.20 (1H, m, H-16a), 3.42 (1H, m, H-16b), 5.34 (1H, dd, $J = 9.9$ and 1.4 Hz, H-18), 3.28 (1H, dd, $J = 11.7$ and 1.4 Hz, H-19a), 3.60 (1H, dd, $J = 11.7$ and 9.9 Hz, H-19b), 1.92 (3H, s, H₃-21), 0.95 (3H, d, $J = 6.6$ Hz, H₃-22), 7.72 (1H, dd, $J = 8.4$ and 1.5 Hz, H-3'), 7.42 (1H, brd, $J = 7.3$ Hz, H-4'), 7.54 (1H, dd, $J = 7.3$ and 1.5 Hz, H-5'), 7.42 (1H, brd, $J = 7.3$ Hz, H-6'), 7.72 (1H, dd, $J = 8.4$ and 1.5 Hz, H-7'), 3.35 (3H, s, O-CH₃); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 166.5$ (qC, C-1), 118.3 (CH, C-2), 157.9 (qC, C-3), 32.1 (CH₂, C-4), 30.1 (CH₂, C-5), 131.9 (CH, C-6), 125.2 (CH, C-7), 127.3 (CH, C-8), 136.2 (CH, C-9), 29.8 (CH, C-10), 30.8 (CH₂, C-11), 30.7 (CH₂, C-12), 63.2 (CH₂, C-13), 35.1 (CH₂, C-14), 66.7 (CH, C-15), 32.2 (CH₂, C-16), 100.0 (CH, C-17), 58.2 (CH, C-18), 28.9 (CH₂, C-19), 171.9 (qC, C-20), 25.2 (CH₃, C-21), 21.8 (CH₃, C-22), 169.7 (qC, C-1'), 133.8 (qC, C-2'), 129.9 (CH, C-3'), 133.2 (CH, C-4'), 128.1 (CH, C-5'), 133.2 (CH, C-6'), 129.9 (CH, C-7'), 48.2 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\text{max}} = 3693$, 3531, 2928, 2856, 2356, 1684, 1453, 1351, 1278, 1149 cm^{-1} ; HRMS-ESI: m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{25}\text{H}_{37}\text{NO}_5\text{SNa}$, 562.2239, found: 562.2234.

17-O-Methyl-N-(7'-methoxyphenylacetyl)latrunculin A (11)—Compound **11** was prepared according to procedure B from **2** (10 mg, 0.022 mmol), in THF (1 mL), NaH (2 mg, 0.041 mmol) in THF (1 mL) and *p*-methoxyphenylacetyl chloride (0.1 mL, 0.636 mmol), elution with *n*-hexane/EtOAc (8:2) afforded **11** (2.2 mg, 22.0%): Colorless oil, $[\alpha]_D^{25}$

+63.6 ($c = 0.1$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.61$ (1H, brs, H-2), 1.91 (1H, m, H-4a), 2.13 (1H, m, H-4b), 2.25 (2H, m, H-5), 5.77 (1H, dt, $J = 15.4$ and 5.8 Hz, H-6), 5.34 (1H, dd, $J = 15.7$ and 10.3 Hz, H-7), 6.00 (1H, dd, $J = 10.6$ and 10.6 Hz, H-8), 4.97 (1H, dd, $J = 10.6$ and 10.4 Hz, H-9), 2.76 (1H, m, H-10), 1.59 (2H, m, H-11), 1.11 (1H, m, H-12a), 1.83 (1H, m, H-12b), 4.01 (1H, m, H-13), 1.47 (1H, m, H-14a), 1.88 (1H, m, H-14b), 5.03 (1H, brt, $J = 3.1$ Hz, H-15), 2.22 (2H, m, H-16), 5.30 (1H, d, $J = 9.0$ Hz, H-18), 3.39 (1H, dd, $J = 12.1$ and 4.0 Hz, H-19a), 3.45 (1H, dd, $J = 12.3$ and 9.1 Hz, H-19b), 1.91 (3H, s, H₃-21), 0.98 (3H, d, $J = 6.2$ Hz, H₃-22), 3.79 (2H, s, H-2'), 7.21 (1H, d, $J = 8.8$ Hz, H-4'), 6.81 (1H, d, $J = 8.8$ Hz, H-5'), 6.81 (1H, d, $J = 8.8$ Hz, H-7'), 7.21 (1H, d, $J = 8.8$ Hz, H-8'), 3.77 (3H, s, H-9'), 3.30 (3H, s, O-CH₃); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 166.3$ (qC, C-1), 118.4, (CH, C-2), 158.9 (qC, C-3), 32.3 (CH₂, C-4), 30.9 (CH₂, C-5), 131.8 (CH, C-6), 125.1 (CH, C-7), 127.4 (CH, C-8), 136.4 (CH, C-9), 29.8 (CH, C-10), 31.9 (CH₂, C-11), 31.7 (CH₂, C-12), 63.0 (CH, C-13), 34.7 (CH₂, C-14), 66.9 (CH, C-15), 33.1 (CH₂, C-16), 101.2 (qC, C-17), 57.6 (CH, C-18), 25.3 (CH₂, C-19), 171.4 (qC, C-20), 25.2 (CH₃, C-21), 21.8 (CH₃, C-22), 175.7 (qC, C-1'), 42.3 (CH₂, C-2'), 126.3 (qC, C-3'), 130.8 (CH, C-4'), 114.0 (CH, C-5'), 159.8 (qC, 6'), 114.0 (CH, C-7'), 130.8 (CH, C-8'), 55.4 (CH₃, C-9'), 48.2 (CH₃, O-CH₃) ppm; IR (neat): $\nu_{\text{max}} = 3693, 3601, 2928, 2855, 2360, 1694, 1602, 1291, 1090\text{ cm}^{-1}$; HRMS-ESI: m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{25}\text{H}_{37}\text{NO}_5\text{SNa}$, 606.2501, found: 606.2500.

N-Ethyllatrunculin A (12)—Compound **12** was prepared according to procedure C starting with **6** (5 mg, 0.011 mmol) in the acidic solution (1 mL); 3 h at 60 °C. Elution with *n*-hexane/EtOAc (9:1) afforded **12** (1.3 mg, 26.0%): colorless oil, $[\alpha]_{\text{D}}^{25} +63.6$ ($c = 0.11$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.69$ (1H, brs, H-2), 2.64 (1H, m, H-4a), 2.76 (1H, m, H-4b), 2.28 (2H, m, H-5), 5.73 (1H, dt, $J = 15.4$ and 4.0 Hz, H-6), 6.41 (1H, dd, $J = 15.0$ and 11.0 Hz, H-7), 5.97 (1H, dd, $J = 10.8$ and 11.0 Hz, H-8), 5.01 (1H, dd, $J = 10.4$ and 10.0 Hz, H-9), 2.86 (1H, m, H-10), 1.11 (1H, m, H-11a), 1.76 (1H, m, H-11b), 1.46 (2H, m, H-12), 4.25 (1H, m, H-13), 1.45 (1H, m, H-14a), 1.80 (1H, m, H-14b), 5.44 (1H, brt, $J = 3.3$ Hz, H-15), 1.79 (1H, dd, $J = 15.4$ and 4.4 Hz, H-16a), 2.14 (1H, m, H-16b), 3.83 (1H, dd, $J = 11.7$ and 2.2 Hz, H-18), 3.35 (1H, dd, $J = 12.1$ and 2.2 Hz, H-19a), 3.49 (1H, dd, $J = 12.1$ and 2.6 Hz, H-19b), 1.93 (3H, s, H₃-21), 1.01 (3H, d, $J = 6.6$ Hz, H₃-22), 3.45 (1H, dq, $J = 17.1$ and 7.0 Hz, H-1'a), 3.76 (dq, $J = 17.1$ and 7.0 Hz, H-1'b), 1.18 (3H, t, $J = 7.0$ Hz, H-2'), 4.01 (1H, s, OH); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 167.4$ (qC, C-1), 118.0 (CH, C-2), 158.7 (qC, C-3), 32.7 (CH₂, C-4), 30.3 (CH₂, C-5), 132.7 (CH, C-6), 125.0 (CH, C-7), 127.8 (CH, C-8), 135.5 (CH, C-9), 29.3 (CH, C-10), 31.4 (CH₂, C-11), 31.3 (CH₂, C-12), 63.6 (CH, C-13), 35.2 (CH₂, C-14), 66.6 (CH, C-15), 35.4 (CH₂, C-16), 101.0 (qC, C-17), 60.5 (CH, C-18), 25.4 (CH₂, C-19), 173.5 (qC, C-20), 25.2 (CH₃, C-21), 21.7 (CH₃, C-22), 40.6 (CH₂, C-1'), 29.9 (CH₃, C-2') ppm; IR (neat): $\nu_{\text{max}} = 3698, 3606, 3021-2856, 2360, 1660, 1602, 1289, 1089\text{ cm}^{-1}$; HRMS-ESI: m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{25}\text{H}_{37}\text{NO}_5\text{SNa}$, 472.2134, found: 472.2133.

N-Benzylatrunculin A (13)—Compound **12** was prepared according to procedure C from **9** (9 mg, 0.0176 mmol) in the acidic solution (1 mL); 5 h at 60 °C. Elution with *n*-hexane/EtOAc (9:1) afforded **12** (1.8 mg, 20.0%): colorless oil, $[\alpha]_{\text{D}}^{25} +63.6$ ($c = 0.17$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.68$ (1H, brs, H-2), 1.91 (1H, m, H-4a), 3.38 (1H, dt, $J = 13.6$ and 11.0 Hz, H-4b), 2.25 (2H, m, H-5), 5.75 (1H, dt, $J = 13.0$ and 5.1 Hz, H-6), 6.36 (1H, dd, $J = 15.4$ and 11.0 Hz, H-7), 5.97 (1H, dd, $J = 10.8$ and 10.6 Hz, H-8), 5.01 (1H, dd, $J = 11.4$ and 10.6 Hz, H-9), 2.90 (1H, m, H-10), 0.89 (1H, m, H-11a), 1.75 (1H, m, H-11b), 1.44 (1H, m, H-12a), 1.67 (1H, m, H-12b), 4.27 (1H, m, H-13), 1.36 (1H, m, H-14a), 1.89 (1H, m, H-14b), 5.42 (1H, brt, $J = 3.0$ Hz, H-15), 1.48 (1H, m, H-16a), 2.02 (1H, m, H-16b), 3.88 (1H, dd, $J = 9.2$ and 2.9 Hz, H-18), 3.33 (1H, dd, $J = 11.0$ and 3.0 Hz, H-19a), 3.66 (1H, dd, $J = 11.0$ and 2.6 Hz, H-19b), 1.90 (3H, s, H₃-21), 1.01 (3H, d, $J = 6.2$

Hz, H₃-22), 4.43 (1H, d, J = 15.0 Hz, H-1'a), 5.15 (1H, d, J = 15.0 Hz, H-1'b), 7.26 (1H, m, H-3'), 7.34 (1H, dd, J = 7.3 and 1.4 Hz, H-4'), 7.31 (1H, m, H-5'), 7.34 (1H, dd, J = 7.3 and 1.4 Hz, H-6'), 7.26 (1H, m, H-7'), 3.91 (1H, s, OH); ¹³C NMR (75 MHz, CDCl₃): δ = 166.7 (qC, C-1), 117.5 (CH, C-2), 158.4 (qC, C-3), 32.1 (CH₂, C-4), 30.5 (CH₂, C-5), 132.0 (CH, C-6), 125.0 (CH, C-7), 127.7 (CH, C-8), 136.4 (CH, C-9), 29.3 (CH, C-10), 31.7 (CH₂, C-11), 30.4 (CH₂, C-12), 63.7 (CH, C-13), 35.0 (CH₂, C-14), 66.9 (CH, C-15), 32.7 (CH₂, C-16), 101.1 (qC, C-17), 62.6 (CH, C-18), 27.1 (CH₂, C-19), 173.8 (qC, C-20), 24.6 (CH₃, C-21), 21.7 (CH₃, C-22), 48.3 (CH₂, C-1'), 136.4 (qC, C-2'), 128.2 (CH, C-3'), 128.7 (CH, C-4'), 127.4 (CH, C-5'), 128.7 (CH, C-6'), 128.2 (CH, C-7') ppm; IR (neat): ν_{max} = 3693, 3567, 2996-2855, 2360, 2337, 1663, 1456, 1280, 1091 cm⁻¹; HRMS-ESI: m/z [M+Na]⁺ calcd for C₂₅H₃₇NO₅Na, 534.2290, found: 534.2304.

N-Hydroxymethylatrunculin A (14)—A solution of 10 mg of **1** in 3 mL EtOH was treated with 3 mL 35% aq. CH₂O solution and stirred for 24 h at 60 °C. 17 Brine solution (5 mL) was then added and the mixture was extracted with CHCl₃ (2 × 5 mL). The residue was chromatographed over Si gel 60 using *n*-hexane/EtOAc (8:2) solvent system to afford **14** (2.8 mg, 28%): colorless oil, $[\alpha]_{\text{D}}^{25} +70$ (c = 2.2 in CHCl₃); 3400 br, 2900, 1670; ¹H NMR (400 MHz, CDCl₃): δ = 5.70 (1H, brs, H-2), 2.97 (1H, m, H-4), 2.03 (1H, m, H-5), 5.75 (1H, dt, J = 15 and 5.6 Hz, H-6), 6.40 (1H, dd, J = 15.5 and 11 Hz, H-7), 5.99 (1H, dd, J = 10.8 and 10.6 Hz, H-8), 5.01 (1H, dd, J = 10.6 and 10.4 Hz, H-9), 2.71 (1H, m, H-10), 1.05 (1H, m, H-11a), 1.75 (1H, m, H-11b), 1.42 (2H, m, H-12), 4.38 (1H, m, H-13), 1.48 (1H, m, H-14a), 1.72 (1H, m, H-14b), 5.42 (1H, brs, H-15), 1.82 (1H, m, H-16a), 2.05 (1H, m, H-16b), 3.88 (1H, dd, J = 7.3 and 7.3 Hz, H-18), 3.33 (1H, dd, J = 12 and 9.5 Hz, H-19a), 3.51 (1H, dd, J = 12 and 2.6 Hz, H-19b), 1.93 (3H, s, H₃-21), 1.00 (3H, d, J = 6.5 Hz, H₃-22), 4.43 (1H, s, C-17-OH), 4.65 (1H, dd, J = 11.0 and 5.0 Hz, H-1'a), 5.19 (1H, dd, J = 11.0 and 9.0 Hz, H-1'b), 3.84 (1H, dd, J = 9.0 and 5.0 Hz, CH₂OH); ¹³C NMR (75 MHz, CDCl₃): δ = 165.5 (qC, C-1), 117.5 (CH, C-2), 158.2 (qC, C-3), 32.4 (CH₂, C-4), 30.5 (CH₂, C-5), 132.0 (CH, C-6), 126.1 (CH, C-7), 127.3 (CH, C-8), 136.3 (CH, C-9), 29.2 (CH, C-10), 31.8 (CH₂, C-11), 31.2 (CH₂, C-12), 62.8 (CH, C-13), 35.0 (CH₂, C-14), 67.9 (CH, C-15), 32.1 (CH₂, C-16), 98.5 (qC, C-17), 66.5 (CH, C-18), 28.7 (CH₂, C-19), 175.5 (qC, C-20), 21.6 (CH₃, C-21), 24.5 (CH₃, C-22), 68.9 (CH₂, C-1') ppm; IR (neat): ν_{max} = 3400 br, 2900, 1670.

Pyrene Actin Polymerization Assays

Actin polymerization was performed as described by the manufacturer (Cytoskeleton, Denver, CO). Briefly, 5 mM final concentration of monomeric actin (1:10 pyrene labeled) was incubated on ice for 10 min with the indicated concentrations of latrunculin A derivatives. Samples were then equilibrated 10 min in ELISA plate reader (BioTek, VT), after which polymerization was induced by the addition of KCl, MgCl₂, and ATP. Compounds **1-14** were tested at 0.1, 0.5, 1.0, and 10 μ M and IC₅₀ for each compound was calculated using GraphPad Prism 5.0.

Cell Culture

Breast cancer cell lines, MCF7 and MDA-MB-231 were purchased from ATCC (Manassas, VA). The cell lines were grown in 10% fetal bovine serum (FBS) and RPMI 1640 (GIBCO-Invitrogen, NY) supplemented with 2 mmol/L glutamine, 100 μ g/mL penicillin G, and 100 μ g/mL streptomycin, at 37 °C under 5% CO₂.

Preparation of different dilutions of latrunculin A derivatives for cell culture assays

A stock solution of each latrunculin A derivative was prepared by dissolving the compound in DMSO at 50 mM concentration for MTT and 1 mM for Cultrex assays. About 1 μ L of the former solutions was transferred to 999 μ L of serum free medium to obtain 50 μ M and 1

μM dilutions (0.001% DMSO) for MTT and Cultrex assays, respectively. Serial dilution was then conducted to obtain the desired concentrations.

MTT Proliferation Assay

The growth of MCF7 and MDA-MB-231 cancer cell lines was measured using MTT kit (TACS^{TM}, TREVIGEN®, Inc.).^[20,21] Exponentially growing cells were plated in a 96-well plate at a density of 8×10^3 cells per well, and allowed to attach for 24 h. Complete growth medium was then replaced with 100 μL of RPMI of serum free medium (GIBCO-Invitrogen, NY) containing various doses (50, 10, 1, 0.5, 0.1 μM) of the specific tested compound and culture continued at 37 °C under 5% CO₂. After 72 h of culture, the cells were treated with MTT solution (10 μL /well) at 37 °C for 4 h. The color reaction was stopped by the addition of solubilization/stop solution (100 μL /well), and the incubation at 37 °C continued to completely dissolve the formazan product. Absorbance of the samples was determined at 570 nm with an ELISA plate reader (BioTek, VT). The number of cells per well was calculated against a standard curve prepared by plating various concentration of cells, as determined by hemocytometer, at the start of each experiment. IC₅₀ for each compound was calculated using nonlinear regression (curve fit) of log concentration versus the number of cells/well implemented using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA).

Cultrex® Cell Invasion Assay

Anti-invasive activities were measured using Trevigen's Cultrex® Cell Invasion Assay as previously described.^[22,23] About 50 μL of basement membrane extract (BME) coat was added per well. After incubation for 4 h at 37 °C in a 5% CO₂, 50,000/50 μL of MDA-MB-231 cells in serum free RPMI medium were added per well to top chamber containing the tested compound at desired concentration (0.1, 0.5, 1 μM). About 150 μL of RPMI medium was added to the lower chamber containing 10% FBS and penicillin/streptomycin and using fibronectin (1 $\mu\text{L}/\text{mL}$) and *N*-formyl-met-leu-phe (10 nM) as chemoattractants. Cells were allowed to migrate to lower chamber at 37 °C in CO₂ incubator. After 24 h, top and bottom chambers were aspirated and washed with washing buffer supplemented with the kit. About 100 μL of Cell Dissociation Solution/Calcein-AM solution was added to the bottom chamber and incubated at 37 °C in CO₂ incubator for 1 h. The cells internalize calcein-AM, and the intracellular esterases cleaved the AM moiety to generate free calcein. Fluorescence of the samples was determined at 485 nm excitation, 520 nm emission, using ELISA plate reader (BioTek, VT). The number of cells that have invaded through the BME coat was calculated using a standard curve.

Molecular Modeling

Three-dimensional structure building and all modeling were performed using the SYBYL program package,^[24] version 8.0, installed on DELL desktop workstations equipped with a dual 2.0 GHz Intel® Xeon® processor running the Red Hat Enterprise Linux (version 5) operating system. X-ray crystallographic structural of **1** is available,^[13] which provided a template for modeling other latrunculins. To maintain the active conformation, actin-bounded crystallographic structure of **1** was used as template to sketch other derivatives using SYBYL 8.0. Conformations of each compound were generated using Confort^{TM} conformational analysis. Energy minimizations were performed using the Tripos force field with a distance-dependent dielectric and the Powell conjugate gradient algorithm with a convergence criterion of 0.01 kcal/(mol Å).^[25] Partial atomic charges were calculated using the semiempirical program MOPAC 6.0 and applying the AM1.^[26]

Molecular Docking

SurFlex-Dock program version 2.0 interfaced with SYBYL 8.0 was used to dock the compounds to the active site of actin.^[27,28] SurFlex-Dock employs an idealized active site ligand (protomol) as a target to generate putative poses of molecules or molecular fragments.^[29,30] These putative poses were scored using the Hammerhead scoring function.^[29,30] The program was used to dock the training set molecules into the active site of G-actin. The 3D structure was taken from the brookhaven protein databank (PDB code: 1esv).^[13]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This publication was supported by the NIH Grant Number P20RR16456 from the INBRE Program of the National Center for Research Resources.

References and Notes

1. a) Newman DJ, Crag GM, Holbeck S, Sausville EA. *Curr Cancer Drug Targets*. 2002; 2:279–308. [PubMed: 12470208] b) Peterson JR, Mitchison TJ. *Chem & Biol*. 2002; 9:1275–1285. [PubMed: 12498880] c) Fenteany G, Zhu S. *Curr Topics Med Chem*. 2003; 3:593–616.
2. a) Kashman Y, Groweiss A, Shmueli U. *Tetrahedron Lett*. 1980; 21:3629–3632. b) Groweiss A, Shmueli U, Kashman Y. *Marine Toxins of Latrunculia magnifica*. *J Org Chem*. 1983; 48:3512–3516.
3. Kashman Y, Groweiss A, Lidor R, Blasberger D, Carmely S. *Tetrahedron*. 1985; 41:1905–1914.
4. El Sayed KA, Youssef DTA, Marchetti D. 2006; 69:219–223.
5. El Sayed KA, Khanfar MA, Shallal HM, Muralidharan A, Awate B, Youssef DTA, Liu Y, Zhou YD, Nagle DG, Shah S. *J Nat Prod*. 2008; 71:396–402. [PubMed: 18298079]
6. Coue M, Brenner SL, Spector I, Korn ED. *FEBS Lett*. 1987; 213:316–318. [PubMed: 3556584]
7. Hayot C, Debeir O, van Ham P, van Damme M, Kiss R, Decaestecker C. *Toxicol Appl Pharmacol*. 2006; 211:30–40. [PubMed: 16005926]
8. Okka M, Tian B, Kaufman PL. *Arch Ophthalmol*. 2004; 122:1482–1488. [PubMed: 15477460]
9. Kaufman, PL.; Geiger, B. US Patent. 772,412. 2002.
10. Spector I, Shochet NR, Blasberger D, Kashman Y. *Cell Motil Cytoskel*. 1989; 13:127–144.
11. Hayot C, Farinelle S, De Decker R, Decaestecker C, Darro F, Kiss R, van Damme M. *Int J Oncol*. 2002; 21:417–425. [PubMed: 12118340]
12. Gashet Y, Tournier S, Millar JAB, Hyams JSA. *Nature*. 2001; 412:352–355. [PubMed: 11460168]
13. Morton WM, Ayscough KR, McLaughlin PJ. *Nat Cell Biol*. 2000; 2:376–378. [PubMed: 10854330]
14. Bubb MR, Govindasamy L, Yarmola EG, Vorobiev SM, Almo SC, Somasundaram T, Chapman MS, Agbandje-McKenna M, McKenna R. *J Biol Chem*. 2002; 277:20999–21006. [PubMed: 11932258]
15. Amagata T, Johnson TA, Cichewicz RH, Tenney K, Mooberry SL, Media J, Edelstein M, Valeriote FA, Crews P. *J Med Chem*. 2008; 51:7234–7242. [PubMed: 18942825]
16. Fürstner A, Kirk D, Fenster M, Aïssa C, De Souza D, Nevado C, Tuttle T, Thiel W, Müller O. *Chem A Europ J*. 2006; 13:135–149.
17. Blasberger D, Carmely S, Cojocaru M, Spector I, Shochet NR, Kashman Y. *Liebigs Ann Chem*. 1989; 12:1171–1188.
18. Cooper JA, Pollard TD. 1982; 85:182–211.
19. Zhai L, Zhao P, Panebra A, Guerrero AL, Khurana S. *J Biol Chem*. 2001; 276:36163–36167. [PubMed: 11500485]

20. Mosmann T. J Immunol Methods. 1983; 65:55–63. [PubMed: 6606682]
21. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Cancer Res. 1988; 48:589–601. [PubMed: 3335022]
22. Tamilarasan KP, Kolluru GK, Rajaram M, Indhumathy M, Saranya R, Chatterjee S. BMC Cell Biol. 2006; 7:17–30. [PubMed: 16584574]
23. Borghesani PR, Peyrin JM, Klein R, Rubin J, Carter AR, Schwartz PM, Luster A, Corfas G, Segal RA. Development. 2002; 6:1435–1442. [PubMed: 11880352]
24. Tripos Associates. SYBYL Molecular Modeling Software, version 8.0. Tripos Associates; St. Louis, MO: 2007. <http://www.tripos.com>
25. Clark M, Cramer RD III, van Opdenbosch N. Comput Chem. 1989; 10:982–1012.
26. Stewart JJ. J Comput Aided Mol Des. 1990; 4:1–105. [PubMed: 2197373]
27. Kellenberger E, Rodrigo J, Muller P, Rognan D. Proteins. 2004; 57:225–242. [PubMed: 15340911]
28. Jain AN. J Med Chem. 2003; 46:499–511. [PubMed: 12570372]
29. Welch W, Ruppert J, Jain AN. Chem Biol. 1996; 3:449–462. [PubMed: 8807875]
30. Ruppert J, Welch W, Jain AN. Protein Sci. 1997; 6:524–533. [PubMed: 9070435]

Figure 1.

Time course for actin polymerization in the presence of **1** (A) and **3** (B) at 0.1 and 1.0 μM doses. Polymerization was monitored by increase in fluorescence intensity ($\blacklozenge$: 0 μM , $\blacksquare$: 0.1 μM , $\blacktriangle$: 1 μM).

Figure 2. Anti-invasive activities of latrunculin derivatives (**1-14**) against MDA-MB-231 using Cultrex™ assay kit. Error bars indicate the SEM of n = 3/concentration.

Figure 3. MOLCAD visualization of electrostatic potential of actin binding site docked with **9** using SurFlex-Dock Program. Some non-interacting amino acids were omitted to view the important internal residues.

Figure 4.
Detailed view of docked structure **3** with the corresponding interacting amino acids of actin binding site.

Figure 5.
H-bonding interaction of docked **14** with carboxylate moiety of Asp 157 at optimum distance calculated by SYBYL 8.0.

Figure 6.
Superimposition of crystallographic structure of **1** (blue) with the docked pose structures of **3** (yellow), **9** (green), and **14** (red).

Scheme 1. Semisynthetic transformations of latrunculin A (**1**).^[a]

[a] Reagents and conditions: (a) R-OH, Et₂O-BF₃, rt, (b) 1- NaH, 0 oC, THF, 1 h. 2- R-X, rt, (c) AcOH/H₂O/THF (3:1:1), 60 oC (d) Benzoic anhydride, DMAP, anh CHCl₃, rt, 12 h, (e) 35% aq. CH₂O, EtOH, 24 h, 60 oC.

Table 1

Structure of Latrunculin A Derivatives.

Compound	R ¹	R ²
1	H	H
2	CH ₃	H
3	A	H
4	B	H
5	CH ₃	CH ₃
6	CH ₃	CH ₂ CH ₃
7	CH ₃	c
8	CH ₃	CH ₂ CH ₂ CH ₂ OH
9	CH ₃	d
10	CH ₃	b
11	CH ₃	e
12	H	CH ₂ CH ₃
13	H	d
14	H	CH ₂ OH

Table 2

Pyrene Actin Polymerization Assay of Latrunculin A Derivatives.

compound	Actin polymerization inhibition (%)		IC ₅₀ (nM) ^[a]
	100 nM	1 μ M	
1	30	73	284 $\pm$ 72
2	6	21	9233 $\pm$ 825
3	31	94	184 $\pm$ 48
4	23	67	466 $\pm$ 51
5	11	19	> 10000
6	0	18	> 10000
7	20	49	1049 $\pm$ 271
8	0	21	8548 $\pm$ 574
9	33	67	333 $\pm$ 76
10	17	53	834 $\pm$ 109
11	0	13	> 10000
12	32	70	283 $\pm$ 51
13	24	64	445 $\pm$ 79
14	35	90	210 $\pm$ 54

^[a]IC₅₀ values are determined in nM from nonlinear dose-response curve using GraphPad Prism. The values are the mean $\pm$ SEM. Each experiment was conducted in triplicate.

Table 3

Antiproliferative Activities of Latrunculin A Derivatives.

compound	IC ₅₀ (μM) ^[a]	
	MCF7	MDA-MB-231
1	0.48 ± 0.04	4.19 ± 1.49
2	10.19 ± 1.72	23.42 ± 0.85
3	0.10 ± 0.07	2.71 ± 0.52
4	0.52 ± 0.14	5.05 ± 0.36
5	13.07 ± 3.69	19.93 ± 3.27
6	20.64 ± 2.77	>50.0
7	5.30 ± 1.32	5.07 ± 0.72
8	19.20 ± 3.91	>50.0
9	0.78 ± 0.24	6.82 ± 1.41
10	0.55 ± 0.091	4.48 ± 1.79
11	15.4 ± 1.21	26.17 ± 3.91
12	0.95 ± 0.28	7.19 ± 1.01
13	0.63 ± 0.02	2.72 ± 0.39
14	0.12 ± 0.01	2.99 ± 0.15

^[a] IC₅₀ values are determined in μM from nonlinear dose-response curve using GraphPad Prism. The values are the mean ± SEM. Each experiment was conducted in triplicate.