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Latrunculin with a Highly Oxidized Thiazolidinone Ring: Structure Assignment and Actin Docking

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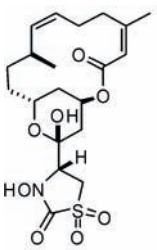
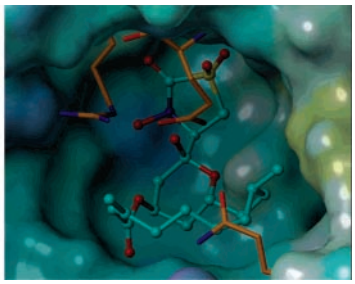
ABSTRACT



Oxalatrunculin B



A new latrunculin, oxalatrunculin B (3), was isolated from Red Sea sponge *Negombata corticata*. Extensive spectroscopic analysis revealed an unprecedented heterocycle in which the rare thiazolidinone ring found in latrunculins was oxidized with three additional oxygens. An actin polymerization inhibition assay agreed with MM-PBSA free energy calculations that 3 binds more weakly than latrunculin B to actin. Significant antifungal and anticancer activity of 3 was found, suggesting an alternate target in addition to actin for latrunculin bioactivity.

Latrunculins A (**1**) and B (**2**) (Figure 1) were first isolated from *Negombata magnifica* (Keller) (formerly *Latrunculia magnifica*), discovered from the Red Sea.[1] Among the characteristic features of these natural products is the presence of a macrocyclic lactone ring of 14 or 16 carbon atoms and a 2-thiazolidinone moiety (Figure 1).[2,3] In vitro experiments revealed that the latrunculins disrupt actin polymerization,[4] a possible target for cancer.[5]

A recent study has also reported antimigratory and antiangiogenic activity of latrunculins, adding to their possible utility in the control of cancer.[6] In addition to significant ichthyotoxic and cytotoxic properties, the latrunculins are also antiviral against herpes simplex type 1 virus (HSV-1).[7]

Several new latrunculins including natural, synthetic, or semisynthetic analogues have been reported recently.[6,8] We

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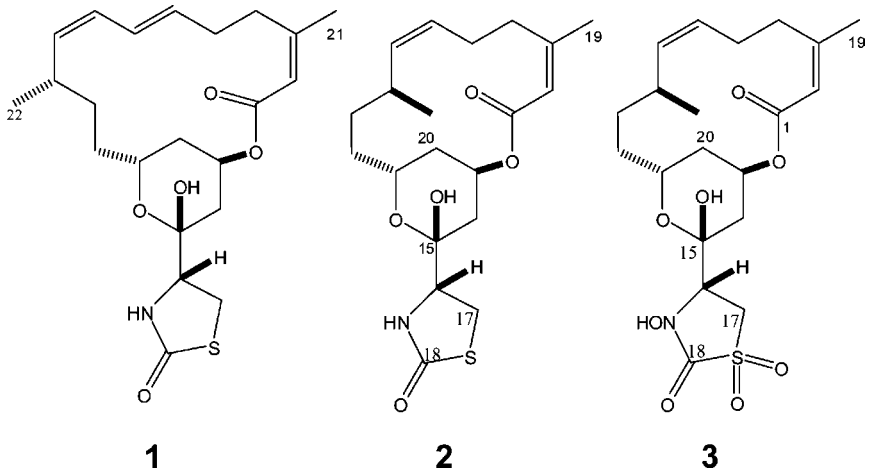

Figure 1. Latrunculins A (**1**) and B (**2**) and oxalatrunculin B (**3**).

report here the structure assignment and bioactivity of a unique new latrunculin, oxalatrunculin B (**3**) which possesses a novel and highly oxidized 2-thiazolidinone ring. Molecular modeling of **1**−**3** in the actin monomer active site was utilized as a predictor of biological activity.

The Red Sea sponge *N. corticata* was extracted with MeOH/CH_2Cl_2. Silica column chromatography of the extract afforded several fractions, one of which yielded metabolite **3**. HRTOF-MS of **3** displayed a molecular ion peak at *m*/*z* = 442.1551 $[M - H]^-$, supporting the molecular formula $C_{20}H_{29}SO_8N$. Comparison of **3** with **2** revealed an increase of 48 mass units. Addition of three oxygen atoms satisfied the formula difference, giving a calculated mass of *m*/*z* = 442.1536 $[M - H]^-$. As further evidence for this, combustion analysis on **3** showed 53.6% carbon, 6.51% hydrogen, 3.16% nitrogen, 28.6% oxygen, and 6.34% sulfur. ^{13}C NMR indicated that the carbon skeleton of **3** consisted of two methyl carbons, seven methylenes, seven methines, and four quaternary carbon atoms, which is identical to the carbon multiplicities of **2**. The ^{13}C NMR chemical shifts for the 14-membered and tetrahydropyran rings were in close agreement with those of **2**, revealing that additional oxygenation must occur at the heteroatoms. Significant differences were found in the chemical shifts of the thiazolidinone ring. The C-17 resonance shifted downfield from δ 28.7 to 49.6, indicating the introduction of a neighboring oxygen atom. The C-16 shifted slightly upfield, so the only possible position for oxidation was at the sulfur atom. The C-18 also moved upfield from δ 175.3 to 157.3. The chemical shift of the carbonyl carbon of a five-membered lactam ring will move upfield around 15 ppm when the lactam nitrogen is hydroxylated.[9,10] It has also been shown that oxygenation of the sulfur atom adjacent to the carbonyl of a five-membered thiazolidinone will move chemical shifts upfield by ~10 ppm.[11] This suggested that two of the three new oxygen atoms are at the S and the last is assigned as the hydroxyl group at N. Further evidence for this was the disappearance of the H−N at δ 6.1. In addition, the amide IR resonance in **2** is absent in **3**, and a new resonance at 1756 cm^{-1} appeared, indicating the presence of a CONOH system.[11] The OH band at 3448 cm^{-1}

Table 1. ^{13}C (100 MHz) and ^1H COSY (400 MHz) and HMBC (in MeOD) NMR Spectral Data (H to C)

No.	δ_C (**2**)	δ_C (**3**)	δ_H (**3**)	*J* (Hz)	HMBC
1	165.6	166.3			
2	118.0	117.9	5.60 (s)		
3	154.7	157.9			
4	35.8	35.9	1.97 (m)		
			3.05 (m)		
5	26.9	26.4	2.15 (m)		
			2.70 (m)		
6	127.6	128.1	5.40 (ddd)	11.2, 11.2, 3.0	
7	135.9	135.2	5.00 (dd)	10.8, 10.8	
8	28.9	28.7	2.70 (m)		
9	31.2	31.0	1.10 (m)		
			1.70 (m)		
10	31.2	34.6	1.70 (m)		
			1.70 (m)		
11	62.6	65.2	4.50 (m)		13
12	35.4	30.7	1.40 (m)		11
			1.60 (m)		
13	68.7	67.5	5.20 (m)		11,15
14	31.8	34.2	2.15 (m)		15,16
			2.44 (d)	15.2	
15	97.7	104.3			
16	61.8	57.8	4.00 (m)		14, 15, 18, 17
17	28.7	49.6	3.00 (m)		15, 16, 18
			3.20(d)	14.4	
18	175.3	157.3			
19	24.1	24.8	1.96 (s)		
20	22.3	21.3	0.90(d)	6.0	

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is significantly increased in intensity for **3**, indicating the presence of two OH groups in **3** versus one OH group in **2**. The IR showed a strong band at 1051 cm^{-1}, indicating the presence of a SO_2 moiety.[12] Inclusion of three oxygen atoms on the thiazolidinone moiety increases its electronegativity and explains the downfield shift of C-15 from δ 97.7 to 104.3. HMBC showed important ^3J correlations of H-16 with C-18, C-14, and C-15 and of H-17 with C-18 and C-15 (Table 1).

We used molecular modeling to calculate the binding affinity of **3** in G-actin. X-ray crystal structures of **1** bound to G-actin, such as 1RDW[13] (Figure 2) showed **1**'s binding

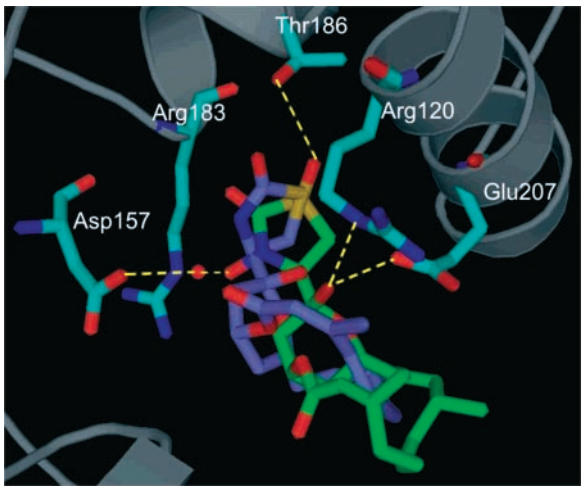

Figure 2. Overlay of **3** (green C, from an MD snapshot), displaced away from the active site compared to **1** (purple C, from 1RDW), in G-actin (cyan C). Hydrogen bonding interactions of **3** are shown (yellow dashed lines), including to a crystallographic water (red O).

site, but there is no X-ray structure available for other latrunculins bound to actin.

Latrunculins **1** and **2** each docked into 1RDW with a highly similar pose to that of the X-ray structure of **1** in actin, as also found in the docking studies of Fürstner et al.[8] Oxalatrunculin **3** docked in a similar pose to that of **1** and **2**, but despite the two extra polar functional groups in **3**, the docking scores (Table 2) predicted poor binding of **3** with actin, with lower GOLD and Chem scores and poorer estimated ΔG. Since, in docking, the receptor is fixed, we proceeded to carry out molecular dynamics (MD) simulations and binding free energy calculations using the MM-PBSA/GBSA method implemented in AMBER 8.0,[14] in water with the entire protein flexible. The major favorable contributions

Table 2. Docking Scores and Binding Energies (ΔE) and Free Energies (ΔG) for Latrunculins **1**−**3** (energies in kcal/mol)

	Molecular Docking			Molecular Dynamics Simulations				
	Gold[a]	Chem[a]	ΔG^b	ΔE_{ele}	ΔE_{vw}	ΔG_A	ΔG^c	ΔG^d
1	90.7	36.4	−37.5	−54.9	−52.9	−6.68	−43.2	−35.5
2	70.0	34.5	−38.6	−40.6	−40.6	−6.19	−26.6	−21.4
3	58.6	20.3	−31.9	−36.8	−39.4	−5.92	−22.3	−14.9

[a] Docking scores. [b] Estimate from ChemScore. [c] MM-GBSA. [d] MM-PBSA.

to binding were Van der Waals and electrostatic terms (Table 2). In the MD simulations, a H-bond to Glu207 was similar for **1**−**3** and was persistently maintained. In the different heterocycle of **3**, partial displacement of the macrocyclic ring of **3** away from the active site and smaller macrocycle ring size compared to **1** (Figure 2) resulted in **3**'s decreased electrostatic and Van der Waals energies. **3** showed water-mediated H-bonding of NOH with Asp157, whereas, for **1**, the NH···Asp157 interaction does not need water assistance (1RDW). Our theoretically most reliable result, from MM-PBSA,[14] reveals a poorer binding free energy for **3** compared to that for **1** or **2**, by 20.6 or 6.5 kcal/mol, respectively.

In an assay designed to measure inhibition of actin polymerization, the EC_{50} of **2** was determined to be 2.56 μM, which is consistent with previous empirical evidence reporting comparisons with **1**.[4] A relative comparison of the percent inhibition of actin polymerization by **2** and **3** showed that **3** had significantly less potency. This matched well with the molecular modeling results, in which **3** binds much more weakly than **2** (Figure 3).

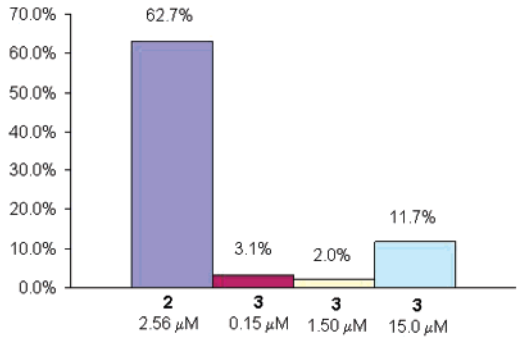

Figure 3. Relative inhibition (DMSO control) of actin polymerization by latrunculin B (**2**) at the predetermined EC_{50} (μM) compared to various concentrations (μM) of the oxalatrunculin B (**3**) analogue. Some inhibition is indicated at the highest concentration tested (15 μM).

Antifungal activity of **3** against *Saccharomyces cerevisiae* NRRL Y-2034 showed an MIC of 56 versus 127 μM for **2**. Cytotoxicity of **3** was evaluated against several cell lines including HepG2, HCT-116, and 1301. **3** showed nonspecific cytotoxicity against solid tumor cells and hematopoietic

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cancerous cells. **3** was also found to be more potent against hepatocellular carcinoma than **2** or latrunculin T, with MIC of 16.34 μM for **3**, 19.27 μM for **2**, and 34.72 μM for latrunculin T. Considering the evidence presented above that **3** binds more weakly than **2** to actin, the stronger cytotoxic activity of **3** compared to that of **2** suggests that there is an alternate target to actin for latrunculin bioactivity or the compound is reduced in situ and acts as a prodrug.

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Supporting Information Available: Experimental procedures, characterization data, computational procedures, and copies of IR, ^{1}H NMR, ^{13}C NMR, HMBC, and HRTOF-MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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