Bitungolides A−F, New Polyketides from the Indonesian Sponge *Theonella* cf. *swinhoei*

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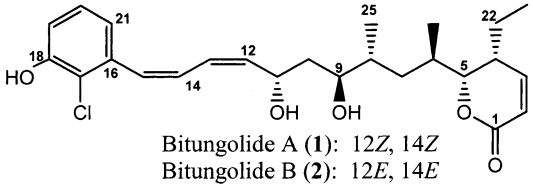
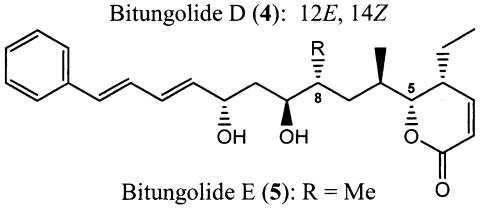
Six new polyketides, bitungolides A−F (**1**−**6**), have been isolated from the Indonesian sponge *Theonella* cf. *swinhoei* and their structures elucidated by spectroscopic data and X-ray diffraction analysis. The bitungolides are a new class of *Theonella* metabolites that inhibit dual-specificity phosphatase VHR.

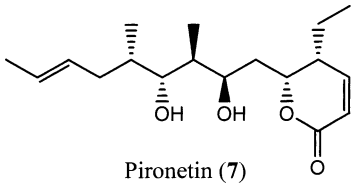
The sponge *Theonella swinhoei* Gray has yielded a variety of metabolites encompassing macrolides[1] and cyclic peptides.[2] Recently we reported cyclic peptides, barangamides, from this species collected in Indonesia.[3] In our continuing collaborative research on bioactive compounds from Indonesian marine organisms, we collected a specimen whose morphology was similar to *T. swinhoei*. In this report we describe the isolation and structure elucidation of novel polyketides, bitungolides A−F (**1**−**6**), showing inhibition of a phosphatase, from *Theonella* cf. *swinhoei* (family Theonellidae).



Bitungolide A (**1**): 12*Z*, 14*Z*
Bitungolide B (**2**): 12*E*, 14*E*
Bitungolide C (**3**): 12*Z*, 14*E*
Bitungolide D (**4**): 12*E*, 14*Z*



Bitungolide E (**5**): R = Me
Bitungolide F (**6**): R = H



Pironetin (**7**)

Results and Discussion

A specimen of the sponge collected in North Sulawesi, Indonesia, was air-dried on site, transported to Okinawa, and extracted by steeping in MeOH. After concentration the residue of the extract was partitioned between CH_2Cl_2 and water to give an oil. The oil was chromatographed on silica gel followed by purification by HPLC or TLC to give six new polyketides, bitungolides A−F (**1**−**6**).

Bitungolide A (**1**) showed pseudomolecular ions at *m*/*z* 447 ([M − H]$^-$) and 449 (3:1) in negative ion FABMS, indicating the presence of a chlorine atom. The molecular formula $C_{25}H_{33}ClO_5$ was determined by HRFABMS (Δ +2.8 mmu). Its IR and NMR spectra suggested the presence of an unsaturated lactone, two secondary hydroxyl groups, two secondary methyls, and an ethyl together with 12 sp^2 carbons. Analysis of the 2D NMR data allowed us to assign the sp^2 signals to a 1,2,3-trisubstitued benzene, a diene, and a double bond conjugated to the lactone carbonyl. The geometry of both double bonds in the diene was shown to be *Z* by coupling constants ($J_{12,13}$ = 11.5 Hz, $J_{14,15}$ = 11.3 Hz). The gross structure of **1** was secured by connecting these structural units with the remaining portion of the molecule by 2D NMR analysis. Assignment of the NMR data is shown in Table 1. Confirmation of the structure was provided by a single crystal X-ray diffraction study which simultaneously determined the absolute stereochemistry of **1** (Figure 1).[4]

Bitungolide B (**2**) had the same molecular formula as **1** as shown by HRFABMS. It exhibited similar NMR signals (Tables 2 and 3). A major difference was found with the diene signals displaying larger coupling constants ($J_{12,13}$ = 15.3 Hz, $J_{14,15}$ = 15.5 Hz). Thus, the diene should have 12*E* and 14*E* configuration. The rest of the molecule was shown to be the same as **1** by detailed NMR analysis. The NMR data also indicated that **2** had the same relative configurations as **1** at all chiral centers. The stereochemical difference in the chromophores of **1** and **2** is reflected in their UV absorptions (λ_{max} 270 and 291 nm), which revealed a bathochromic shift from **1** to **2**. Because of the steric hindrance inherent in the 14*Z* configuration, the diene moiety and the aromatic ring in **1** are not coplanar, as seen in the X-ray structure (Figure 1), while the less hindered 14*E* configuration in **2** should give more efficient conjugation of the chromophore.

Bitungolides C (**3**) and D (**4**) were also shown to have the same molecular formulas $C_{25}H_{33}ClO_5$ as **1** and **2**. The diene portion of **3** was 12*Z* and 14*E* ($J_{12,13}$ = 11.3 Hz, $J_{14,15}$ = 15.3 Hz), while that of **4** was 12*E* and 14*Z* ($J_{12,13}$ = 15.3 Hz, $J_{14,15}$ = 11.3 Hz). The remaining portions were confirmed to be the same as those of **1** and **2** by mainly 2D

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Table 1. ^{1}H and ^{13}C NMR Data for Bitungolide A (**1**) in $CDCl_3$

no.	^{13}C NMR[a]	^{1}H NMR [mult., *J* (Hz)][b]	COSY	HMBC (H→C)
1	164.9 s			
2	120.9 d	6.05 d, 9.8	3	1, 4
3	151.2 d	7.09 dd, 9.8, 6.4	2, 4	1, 4, 5
4	36.7 d	2.36 m	3, 5, 22ab	
5	85.0 d	3.98 dd 10.4, 3.0	4, 6	6, 22
6	31.0 d	1.96 m	5, 7ab, 24	
7	35.2 t	a 1.80 m	6, 7b	8, 24, 25
		b 1.23 brd, 10.0	6, 7a	6, 8, 9, 24, 25
8	36.1 d	1.79 m	9, 25	25
9	73.2 d	3.81 br	8, 10ab, 9-OH	
9-OH		2.45 br	9	
10	39.5 t	a 1.79 m	9, 11	9, 11, 12
		b 1.72 m	9, 11	9, 11, 12
11	66.0 d	5.05 br	10ab, 11-OH, 12	
11-OH		2.47 br	11	10, 11
12	136.3 d	5.68 ddt, 11.5, 8.5, 1.5	11, 13	14
13	125.0 d	6.34 t, 11.5	12, 14	11, 14, 15
14	126.0 d	6.67 dd, 11.5, 11.3	13, 15	12, 13, 15, 16
15	128.4 d	6.58 d, 11.3	14	13, 14, 17, 21
16	135.6 s			
17	119.4 s			
18	151.6 s			
18-OH		5.71 brs		17, 18, 19
19	115.0 d	6.96 dd, 8.0, 1.2	20	17, 18, 21
20	127.1 d	7.16 t, 8.0	19, 21	16, 17, 18, 19
21	123.0 d	6.93 dd, 8.0, 1.2	20	15, 17, 19
22	20.1 t	a 1.69 m	4, 22b, 23	3, 4, 23
		b 1.49 m	4, 22a, 23	3, 4, 23
23	11.0 q	0.97 t, 7.6	22a, 22b	4, 22
24	14.7 q	0.89 d, 6.7	6	5, 6, 7
25	14.7 q	0.92 d, 6.4	8	7, 8, 9

[a] $CDCl_3$ (δ 77.1) signal was used as internal standard for ^{13}C NMR (125 MHz). [b] TMS (δ 0.00) signal was used as internal standard for ^{1}H NMR (500 MHz).

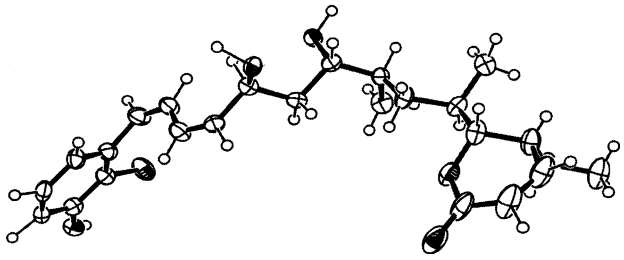


Figure 1. Computer-generated ORTEP drawing of bitungolide A (**1**).

NMR analysis (Tables 2 and 3). Compounds **1**−**4** are geometric isomers, some of which may be artifacts formed by photoisomerization during sample handling, e.g., air-drying of the sponge.

The molecular formula of bitungolide E (**5**), $C_{25}H_{34}O_4$ (Δ −2.7 mmu), indicated the absence of a chlorine atom and one of the oxygens found in bitungolides A−D. The NMR spectra of **5** showed the presence of a monosubstituted benzene (δ 7.23, 7.31, 7.39; δ 126.3, 127.5, 128.6, 137.2) instead of the 2,3-disubstituted phenol in **1**−**4**, confirming that the difference is on the aromatic portion. Similarity of the NMR signals (Tables 2 and 3) for the remaining portion of the molecule to those of bitungolide B (**2**) and 2D NMR analysis allowed us to depict the structure **5** for bitungolide E.

Bitungolide F (**6**) was analyzed for $C_{24}H_{32}O_4$ (Δ −2.2 mmu) by HRAPCIMS. The NMR spectra of **6** showed two methyls (δ 11.0, 14.9) and four methylenes (δ 20.1, 28.5, 34.5, 42.6) instead of three methyls and three methylenes in **5**, suggesting that **6** is a demethyl derivative of **5**. The position of demethylation in **6** was determined to be at C-8 by taking connectivity for the portion of C-5−C-11 with COSY (H-5/H-6, H-6/H-7b,24, H-7a/H-7b,8ab, H-7b/H-8ab, H-8a/H-8b,9, H-8b/H-9, H-9/H-10ab, and H-10ab/H-11) and HMBC (C-6/H-5,7a,24, C-7/H-5.8ab,24, C-8/H-7b,10ab, C-9/H-8ab,10ab,11, C-10/H-8ab,11,12). Since the remaining NMR signals for the lactone and aromatic moieties were almost identical (Tables 2 and 3) to those of **5**, **6** was elucidated to be 8-demethylbitungolide E.

Bitungolides have a structural feature reminiscent of pironetin (**7**), which has been reported to exhibit antitumor activity by arresting the cell cycle at the M-phase.[5] When bitungolides were assayed against 3Y1 rat normal fibroblast cells, a cytotoxic effect was observed at 10 μg/mL. These compounds did not act on cytoskeletons (microtubules and actin), and no morphological change on nuclei was observed. In assays with phosphatases, they showed weak activity against dual-specificity phosphatase (VHR), while no activity was observed against serine/threonine phosphatase (PP1 and PP2A) or tyrosine phosphatase (PTP-S2) (Figure 2).

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Jasco DIP-1000 polarimeter. IR spectra were recorded on a Jasco FT/IR-300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a JEOL A-500 NMR spectrometer and mass spectra on a JEOL JMS-700 instrument.

Animal Material. The sponge (2.7 kg, dry wt) was collected at a coral reef (−10 to −20 m) along Lembeh Strait off Bitung, Sulawesi Island, Indonesia, in August 1999. The specimen was air-dried under shade and brought to Okinawa. A voucher specimen (QMG318533) has been deposited at the Queensland Museum, South Brisbane, Australia. The sponge was initially identified to be *Theonella swinhoei* by Dr. John N. A. Hooper of the Queensland Museum. However, since it has some different morphological features, i.e., harder texture and smaller area of attachment to substratum, from the specimens of *T. swinhoei* collected in several localities of the Indo-Pacific, we decided to designate the present sample as *Theonella* cf. *swinhoei* after discussion with Dr. Hooper.

Extraction and Isolation. The sample (2.7 kg) was extracted with MeOH three times. After filtration and con-

Table 2. ^{1}H NMR Data for Bitungolide B−F (**2**−**6**) in $CDCl_3$ [a,b]

no.	2	3	4	5	6
2	6.05 d, 9.8	6.05 d, 9.8	6.05 d, 9.8	6.04 dd, 0.6, 9.8	6.04 d, 9.8
3	7.08 dd, 6.4, 9.8	7.08 dd, 6.5, 9.8	7.09 dd, 6.4, 9.8	7.08 dd, 6.4, 9.8	7.08 dd, 6.4, 9.8
4	2.36 m	2.36 m	2.36 m	2.35 m	2.33 m
5	3.97 dd	3.97 dd	3.96 dd	3.95 dd	4.00 dd
	3.0, 10.4	2.8, 10.4	3.1, 10.4	3.1, 10.4	3.1, 10.7
6	1.95 m	1.95 m	1.93 m	1.94 m	1.85 m
7	a 1.80 m	a 1.80 m	a 1.76 m	a 1.79 m	a 1.92 m
	b 1.20 ddd	b 1.22 brdd	b 1.17 ddd	b 1.20 ddd	b 1.29 m
	2.0, 10.0, 13.0	10.4, 12.5	2.4, 10.4, 13.1	2.4, 10.4, 12.5	
8	1.74 m	1.74 m	1.70 m	1.70 m	a 1.68 m; b1.44 m
9	3.80 m	3.82 m	3.75 m	3.79 ddd	3.98 m
				2.1, 5.5, 9.8	
OH	2.47 br	2.42 d, 4.0		2.50 br	
10	a 1.83 m	a 1.75 m	a 1.76 m	a 1.81 m	a 1.80 m
	b 1.72 m	b 1.75 m	b 1.66 m	b 1.70 m	b 1.75 m
11	4.62 m	5.06 m	4.54 m	4.60 m	4.59 m
OH	2.73 brs	2.46 d, 4.0	2.66 d, 4.5	2.70 br	
12	5.96 dd	5.70 dd	5.97 dd	5.90 dd	5.90 dd
	5.8, 15.3	8.2, 11.3	6.0, 15.3	6.1, 15.3	6.1, 15.0
13	6.53 ddd	6.27 t, 11.3	6.62 ddd	6.46 ddd	6.46 brdd
	1.0, 10.4, 15.3		1.0, 11.3, 15.3	0.6, 10.4, 15.3	10.7, 15.0
14	6.78 dd	7.10 dd	6.38 t, 11.3	6.78 dd	6.78 dd
	10.4, 15.5	11.3, 15.3		10.4, 15.6	10.7, 15.5
15	6.88 d, 15.5	6.90 brd, 15.3	6.48 d, 11.3	6.56 brd, 15.6	6.56 d, 15.5
17				7.39 brd, 7.4	7.40 d, 7.5
18				7.31 t, 7.4	7.31 t, 7.5
OH	5.65 brs	5.69 brs	5.78 brs		
19	6.71 dd, 2.0, 8.0	6.93 dd, 1.8, 7.9	6.95 d, 8.0	7.23 tt, 7.4, 1.2	7.22 t, 7.5
20	7.14 m	7.15 t, 7.9	7.16 t, 8.0	7.31 t, 7.4	7.31 t, 7.5
21	7.16 m	7.19 dd, 1.8, 7.9	6.95 d, 8.0	7.39 brd, 7.4	7.40 d, 7.5
22	a 1.69 m	a 1.69 m	a 1.68 m	a 1.69 m	a 1.67 m
	b 1.50 m	b 1.50 m	b 1.50 m	b 1.49 m	b 1.49 m
23	0.96 t, 7.6	0.97 t, 7.6	0.97 t, 7.4	0.96 t, 7.6	0.95 t, 7.6
24	0.89 d, 6.7	0.89 d, 6.7	0.87 d, 7.0	0.88 d, 6.4	0.90 d, 6.7
25	0.91 d, 6.4	0.92 d, 6.7	0.89 d, 7.0	0.91 d, 6.7	

[a] The figures after multiplicity signs denote coupling constants. [b] TMS (δ 0.00) signal was used as internal standard.

Table 3. ^{13}C NMR Data for Bitungolide B−F (**2**−**6**) in $CDCl_3$ [a]

no.	2	3	4	5	6
1	164.9 s	164.9 s	164.9 s	164.9 s	164.9 s
2	120.9 d	120.9 d	120.9 d	120.9 d	120.8 d
3	151.2 d	151.1 d	151.2 d	151.2 d	151.2 d
4	36.7 d	36.7 d	36.7 d	36.7 d	36.5 d
5	85.0 d	85.0 d	85.0 d	85.0 d	84.5 d
6	31.0 d	31.0 d	31.0 d	31.0 d	33.5 d
7	35.2 t	35.2 t	35.2 t	35.2 t	28.5 t
8	36.1 d	36.2 d	36.1 d	36.1 d	34.5 t
9	73.4 d	73.2 d	73.3 d	73.4 d	69.3 d
10	38.7 t	39.7 t	38.7 t	38.8 t	42.6 t
11	70.3 d	66.3 d	70.3 d	70.4 d	70.2 d
12	137.8 d	135.1 d	139.3 d	136.1 d	136.1 d
13	129.9 d	129.4 d	125.8 d	130.3 d	130.3 d
14	131.3 d	126.6 d	131.2 d	128.2 d	128.2 d
15	128.0 d	129.8 d	126.7 d	132.6 d	132.6 d
16	135.8 s	135.7 s	136.0 s	137.2 s	137.2 s
17	119.0 s	119.3 s	119.3 s	126.3 d	126.3 d
18	151.6 s	151.7 s	151.7 s	128.6 d	128.6 d
19	114.7 d	115.1 d	114.8 d	127.5 d	127.5 d
20	127.5 d	127.6 d	127.3 d	128.6 d	128.6 d
21	118.1 d	118.5 d	122.8 d	126.3 d	126.3 d
22	20.1 t	20.2 t	20.2 t	20.1 t	20.1 t
23	11.0 q	11.0 q	11.0 q	11.0 q	11.0 q
24	14.7 q	14.7 q	14.7 q	14.7 q	14.9 q
25	14.6 q	14.7 q	14.5 q	14.6 q	−

[a] $CDCl_3$ (δ 77.1) signal was used as internal standard.

centration, the resulting residue was partitioned between CH_2Cl_2 and water to give 13.50 g (0.50%) of a dark green oil. The oil was first separated by vacuum flash chromatography on silica gel eluting with a step-gradient of hexanes, CH_2Cl_2, EtOAc, and MeOH to furnish 10 fractions. The seventh fraction (1.40 g) was repeatedly separated on silica gel followed by reversed-phase HPLC (MeOH−H_2O, 7:3) to give **1**, **2**, **4**, **5**, and **6**. Similar separation of fractions 8 and 9 gave **3** and an additional amount of the other compounds. Total yields of **1**−**6** were 28.4, 72.2, 2.2, 11.1, 27.4, and 4.0 mg, respectively.

Bitungolide A (1): colorless needles from aqueous MeOH, mp 179−182 °C; $[\alpha]^{27}_D$ +30° (*c* 0.38, MeOH); UV (MeOH) λ_{max} 270 nm (log ϵ 4.1); IR (KBr) 3383, 1699 cm^{-1}; negative ion FABMS *m*/*z* 447, 449 [M − H]$^-$; HRFABMS *m*/*z* 447.1966 ([M − H]$^-$, calcd for $C_{25}H_{32}ClO_5$ 447.1938, Δ +2.8 mmu).

Bitungolide B (2): white amorphous soild, $[\alpha]^{27}_D$ +42° (*c* 4.2, $CHCl_3$); UV (MeOH) λ_{max} 291 nm (log ϵ 4.0); IR (KBr) 3384, 1701 cm^{-1}; FABMS *m*/*z* 447, 449 [M − H]$^-$; HRFABMS *m*/*z* 447.1975 ([M − H]$^-$, Δ +3.7 mmu).

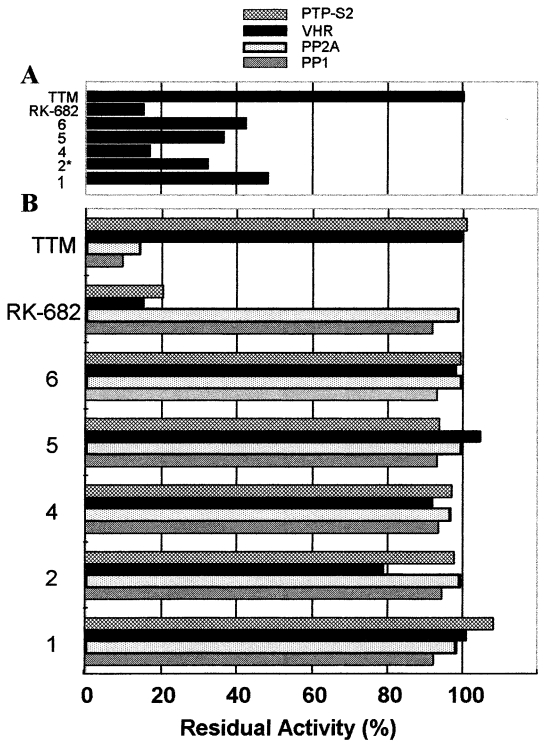
Bitungolide C (3): white amorphous solid, $[\alpha]^{27}_D$ +89° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} 286 nm (log ϵ 4.0); IR (KBr) 3383, 1697 cm^{-1}; FABMS *m*/*z* 447, 449 [M − H]$^-$; HRFABMS *m*/*z* 447.1901 ([M − H]$^-$, Δ −3.7 mmu).

Bitungolide D (4): white amorphous solid, $[\alpha]^{27}_D$ +66° (*c* 0.58, $CHCl_3$); UV (MeOH) λ_{max} 270 nm (log ϵ 4.1); IR (KBr) 3383, 1699 cm^{-1}; FABMS *m*/*z* 447, 449 [M − H]$^-$; HRFABMS *m*/*z* 447.1958 ([M − H]$^-$, Δ +2.0 mmu).

Bitungolide E (5): pale yellow glass, $[\alpha]^{27}_D$ +107° (*c* 1.26, $CHCl_3$); IR (KBr) 3442, 1714 cm^{-1}; APCIMS *m*/*z* 397 [M − H]$^-$; HRAPCIMS *m*/*z* 397.2352 ([M − H]$^-$, calcd for $C_{25}H_{33}O_4$ 397.2379, Δ −2.7 mmu).

Bitungolide F (6): pale yellow glass, $[\alpha]^{27}_D$ +43° (*c* 0.85, $CHCl_3$); IR (KBr) 3421, 1707 cm^{-1}; APCIMS *m*/*z* 383 [M − H]$^-$; HRAPCIMS *m*/*z* 383.2200 ([M − H]$^-$, calcd for $C_{24}H_{31}O_4$ 383.2222, Δ −2.2 mmu).

X-ray Diffraction of 1.[4] Suitable colorless crystals of **1** were obtained by recrystallization from aqueous MeOH. The crystal (0.1 × 0.1 × 0.5 mm) belongs to the orthorhombic system, space group $P2_12_12_1$, with a = 13.6083(8) Å, b = 19.8120(1) Å, c = 9.5455(5) Å, V = 2573.73(2) Å^3, Z = 4, D_{calcd} = 1.097 g/cm^3, λ(Mo Kα) = 0.71069 Å. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to 2θ of 55°. A total of 3325 reflections were collected. The structure was solved by direct methods (SIR 92) and refined by a full



A Assay at concentration of 100 μg/mL; TTM: tautomycin, 30 ng/mL; RK-682:[11] 10 μg/mL; *Assay at 30 μg/mL; VHR: dual-specificity protein phosphatase vaccinia VH1-related.

B Assay at concentration of 10 μg/mL; TTM: 30 ng/mL; PTP-S2: protein tyrosine phosphatase-S2; PP2A: protein phosphatase type 2A; PP1: protein phosphatase type 1.

Figure 2. Effects of bitungolides against phosphatases.

matrix least squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final $R = 0.066$, $R_w = 0.077$ for 1851 observed reflections [$I > 2.00\sigma(I)$] and 281 variable parameters. The Flack parameter was calculated to be 0.0242 (0.00126).

Cell Culture and Cell Proliferation Assay. Rat normal fibroblast 3Y1 cells[6] were grown in Dulbecco's modified MEM culture medium supplemented with 10% fetal calf serum under a humidified atmosphere containing 5% CO_2. Effects of bitungolides on the cell cycle progression and cytoskeletons were observed by flow cytometry[5,7] and indirect immunofluorescence microscopy,[7,8] respectively.

In Vitro Microtubule Assembly Assay. Microtubule assembly was monitored by the turbidity assay as described.[7] In brief, microtubule protein (2.0 mg/mL in Mes buffer) was incubated at 37 °C, and the change in absorbance at 350 nm was monitored with time.

Phosphatase Inhibition Assay.[9] Protein phophatase type 1 (PP1 from rabbit skeletal muscle) and protein phosphatase type 2A (PP2A from human red blood cells) were purchased from Upstate Biotechnology (NY). In vitro protein phosphatase assays were carried out in duplicate as described previously.[8,9] Tyrosine phosphatase PTP-S2 and dual-specificity phosphatase VHR were overexpressed in BL21(DE3), and phosphatase assays were performed in triplicate according to a previous report.[8]

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Supporting Information Available: X-ray structure report on bitungolide A. This material is available free of charge via the Internet at http://pubs.acs.org.

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