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Sesterterpenoids and an alkaloid from a *Thorectandra* sp. as inhibitors of the phosphatase Cdc25B ☆

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Abstract—Bioassay-directed separation of an extract of a *Thorectandra* sp. sponge led to the isolation of three new sesterterpenoids, 16-oxoluffariellolide (**1**), 16-hydroxyluffariellolide (**2**), and (2*E*,6*E*,10*E*)-3-formyl-7,11-dimethyl-13-(2,6,6-trimethylcyclohex-1-enyl)trideca-2,6,10-trienoic acid (**3**); two known sesterterpenoids, luffariellolide (**4**) and dehydroluffariellolide diacid (**5**); and one known alkaloid, fascaplysin (**6**). The structures of the new compounds **1**–**3** were established on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. Compound **6** showed inhibitory activity in the Cdc25B assay, with an IC_{50} value of 1.0 μg/mL.

1. Introduction

During our continuing investigation[1] of marine organisms in the search for natural products with activity against the phosphatase Cdc25B, an extract from a sponge of a *Thorectandra* sp. (family Thorectidae) was found to be active in this assay with an IC_{50} of 8 μg/mL. Sponges of *Thorectandra* sp. have been investigated previously, and have been found to contain a variety of unusual compounds, including furanoditerpenoids,[2] sesterterpenoids,[3–5] and alkaloids.[6,7] Bioassay-directed fractionation led to the isolation of three new (**1**–**3**) and two known (**4**[8,9] and **5**[9,10]) sesterterpenoids and one known alkaloid, fascaplysin (**6**)[8–11]. The inhibitory property of compound **4** against Cdc25 has been reported,[12] and it also showed inhibition of PLA2.[13] The activity of compound **5** in the human 15-lipoxygenase (15-HLO) assay has been evaluated.[14] Fascaplysin (**6**) demonstrated cytotoxic and antimicrobial properties,[7,8,10,15] and exhibited inhibition of CDK4.[16] Compound **6** also showed DNA binding[17] and antimalarial[11] properties, and was tested in the assay against reverse transcriptase.[9] Among compounds **1**–**6**, compound **6** was the most active, with an IC_{50} value of 1.0 μg/mL in the Cdc25B assay. The structures of the known compounds **4**–**6** were identified by comparison of their spectral data with previously published values. In this paper we report the isolation, structure elucidation, and biological activity of the isolates.

2. Results and discussion

16-Oxoluffariellolide (**1**) was obtained as an oil with a molecular formula of $C_{25}H_{36}O_4$ based on a quasimolecular ion peak at *m/z* 401.2691 $[M+H]^+$ in its HRFABMS spectrum. Its ^1H NMR spectrum displayed signals corresponding to five methyls (δ_H 1.17, s, H_3-20; 1.17 s, H_3-21; 1.64 s, H_3-24; 1.67 s, H_3-23; and 1.77 s, H_3-22), three olefinic protons (δ_H 5.87, s, H-2; 5.15, t, J = 6.6 Hz, H-6; and 5.11, t, J = 6.6 Hz, H-10), one methine in the γ-hydroxybutenolide moiety (δ_H 5.98, s, H-25), and eight methylenes (δ_H 1.81–2.46). Its ^{13}C NMR and HSQCED spectra showed signals for 25 carbons, including one ketone (δ_C 199.2, C-16), one carboxyl (δ_C 171.5, C-1), four double bonds (δ_C 168.6, C-3; 165.0, C-14; 137.3, C-7; 134.9, C-11; 130.9, C-15; 124.5, C-10; 122.2, C-6; and 118.0, C-2), and one tertiary carbon in the γ-hydroxybutenolide moiety (δ_C 98.5, C-25); the other fourteen carbons (eight methylenes, five methyls, and one quaternary carbon) had chemical shifts from δ_C 11.6 to δ_C 39.4.

Keywords: Sesterterpenoid; Alkaloid; Phosphatase.
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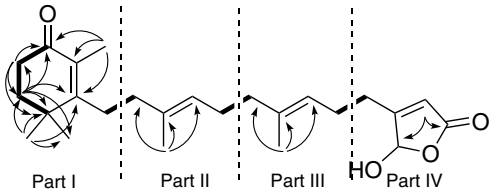
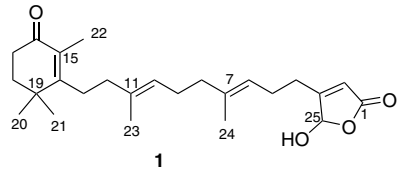


Figure 1. Selected HMBC (arrows) and COSY (bold) correlations for **1**.

On the basis of HSQCED and HMBC spectra, **1** was determined to be composed of four parts, which could be joined together according to the COSY correlations (Fig. 1). Comparison of ^{1}H and ^{13}C NMR data (Table 1) with the literature revealed **1** to be a luffariellolide-type sesterterpenoid bearing one ketone at 16-position.

The ^{13}C NMR chemical shifts of Parts I and II of **1** were similar to those of isodehydroluffariellolide,[2] while Parts III and IV resembled the corresponding fragment of luffariellolide (**4**). The *E*-configurations of the trisubstituted olefins at the 6- and 10-positions were determined by ^{13}C NMR data of the known 3,7-dimethyl-octa-2(*E*-),6-dien-1-ol and 3,7-dimethyl-octa-2(*Z*),6-dien-1-ol].[18] The methyl carbon signals [δ_C 16.0 (C-23); and 16.2 (C-24)] of **1** were coincident with that [δ_C 16.0 (2-CH_3)] of 3,7-dimethyl-octa-2(*E*-),6-dien-1-ol, but not that [δ_C 23.5 (2-CH_3)] of 3,7-dimethyl-octa-2(*Z*),6-dien-1-ol. Therefore, the structure of **1** was determined to be 16-oxoluffariellolide.



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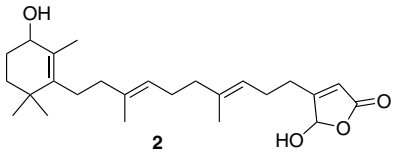
Compound **2** was also obtained as an oil. The ^{1}H NMR and ^{13}C NMR data were closely related to those of **1**, except for additional signals arising from a secondary alcohol (16-position of **2**: δ_H 3.90, δ_C 70.4) instead of a carbonyl signal (16-position of **1**: δ_C 199.2), which suggested that **2** is a reduced product of **1**. The chemical shifts of the 16-position of compound **2** matched those of 3-(2-hydroxyethyl)-2,4,4-trimethylcyclohex-2-enol (1-position: δ_H 3.91, δ_C 70.2), which supported the above deduction.[19] The detailed assignments of the ^{1}H NMR and ^{13}C NMR signals were performed by COSY, HSQCED, and HMBC experiments. Like **1**, the *E* configurations of 6- and 10-positions of **2** were determined on the basis of the comparison of its ^{13}C NMR chemical shifts with those of 3,7-dimethyl-octa-2(*E*-),6-dien-1-ol and 3,7-dimethyl-octa-2(*Z*),6-dien-1-ol.[18] The HRFABMS of compound **2** failed to give a molecular ion, but instead an ion at *m*/*z* 385 [M−OH]; however, the Finnigan LC(−)ESIMS of compound **2** exhibited an ion at *m*/*z* 401 [M−H], which confirmed the molecular formula of compound **2** as $C_{25}H_{38}O_4$. Hence, the structure of **2** was determined to be the 16-hydroxy-luffariellolide. The stereochemistry of the 16- and 25-positions could not be determined due to the lack of material.

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1**–**3**

No	**1**		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		171.5		171.4		173.4
2	5.87 (s)	118.0	5.82 (s)	117.5	6.73 (s)	148.0
3		168.6		169.4		145.4
4	2.46 (t, *J* = 6.8 Hz)	27.7	2.44 (t, *J* = 7.3 Hz)	27.8	2.46 (t, *J* = 7.9 Hz)	26.1
5	2.33 (m)	25.2	2.31 (dt, *J* = 7.3, 6.6 Hz)	25.0	2.10 (m)	28.5
6	5.15 (t, *J* = 6.6 Hz)	122.2	5.09 (t, *J* = 6.6 Hz)	122.4	5.13 (m)	125.0
7		137.3		137.0		136.9
8	2.03 (m)	39.5	2.02 (m)	39.5	1.95–2.08 (m)	40.8
9	2.09 (m)	26.4	2.08 (dt, *J* = 7.3, 6.6 Hz)	26.1	1.95–2.08 (m)	27.6
10	5.11 (t, *J* = 6.6 Hz)	124.5	5.08 (t, *J* = 6.6 Hz)	123.6	5.13 (m)	125.2
11		134.9		135.8		136.7
12	2.09 (m)	38.5	2.02 (m)	39.7	1.95–2.08 (m)	41.6
13	2.30 (m)	30.1	2.02 (m)	28.1	1.95–2.08 (m)	30.8
14		165.0		142.5		138.3
15		130.9		128.4		128.1
16		199.2	3.90 (t, *J* = 4.4 Hz)	70.4	1.91 (t, *J* = 6.2 Hz)	33.8
17	2.46 (t, *J* = 6.8 Hz)	34.3	1.84 (m) 1.66 (m)	28.6	1.58 (m)	20.1
18	1.81 (t, *J* = 6.8 Hz)	37.4	1.59 (m) 1.35 (m)	34.4	1.42 (m)	41.1
19		36.4		35.4		36.0
20	1.17 (s)	26.9	1.04 (s)	27.0	0.99 (s)	29.1
21	1.17 (s)	26.9	0.96 (s)	28.5	0.99 (s)	29.2
22	1.77 (s)	11.6	1.73 (s)	16.9	1.60 (s)	20.6
23	1.67 (s)	16.0	1.63 (s)	15.9	1.63 (s)	16.1
24	1.64 (s)	16.2	1.61 (s)	16.0	1.60 (s)	16.1
25	5.98 (s)	98.5	6.01 (s)	99.4	9.40 (s)	197.9



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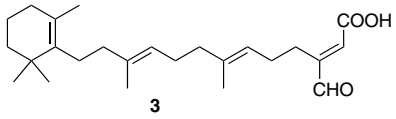
Compound **3** was analyzed as $C_{25}H_{38}O_3$ by HRFABMS and ^{13}C NMR. The only difference between **3** and **4** in their ^{1}H and ^{13}C NMR spectra was the replacement of the methine in the γ-hydroxybutenolide moiety of **4** by an aldehyde group (δ_H 9.40; δ_C 197.9) of **3**, as evidenced by its HMBC spectrum (Fig. 2).

The configurations of the double bonds at C-6(7) and C-10(11) were revealed as *E* like the case of **1**. The stereochemistry of the double bond between carbons 2 and 3 was also established as *E*, based on the ROESY correlation between H-2 and H-25 (Fig. 2). Hence, the structure of **3** was determined as shown.



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The structures of the known compounds **4**–**6** were confirmed by comparison of their ^{1}H and ^{13}C NMR data with the literature data.[8–11]

Luffariellolide-type sesterterpenoids have been reported to be active against Cdc25.[20] Among the five sesterterpenoids isolated in this study, compound **5** showed the most potent inhibitory activity (IC_{50} = 1.6 μg/mL). Fascaplysin (**6**), the only alkaloid obtained from the most active fraction, had an IC_{50} value of 1.0 μg/mL (Table 2). All compounds showed similar inhibitory activity against the catalytic domain and full length Cdc25B, consistent with an inhibitor of the catalytic domain. Concentrations as high as 40 μg/mL of compounds **1**, **2**, **4** and **5** had no inhibitory activity against the closely related dual specificity phosphatase VHR and compounds **4** and **5** had IC_{50} values for the protein tyrosine phosphate PTP1B that were more than 10-fold higher compared to the values for Cdc25B. Insufficient amounts of the other compounds precluded enzymatic analyses of the other compounds. There are no literature reports about the activity of fascaplysin-type alkaloids against Cdc25B, and compound **6** thus represents a new structural type which demonstrates Cdc25B activity. Thus further pharmacological studies on the above-mentioned type of alkaloids as possible Cdc25B-targeted antitumor agents seems warranted.

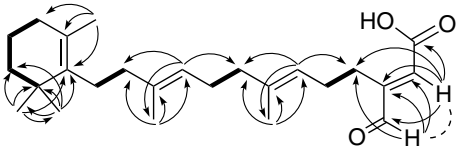
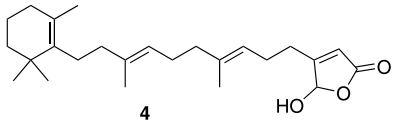


Figure 2. Selected HMBC (arrows), COSY (bold) and ROESY (dashed) correlations for **3**.

Table 2. Activities of compounds **1**–**6** in the Cdc25B assay

Compound	**1**	**2**	**3**	**4**	**5**	**6**
IC_{50} (μg/mL)	17	15	11	33	1.6	1.0



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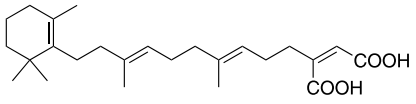

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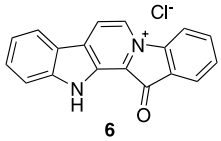

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3. Experimental section

3.1. General experimental procedures

Optical rotations were recorded on a Perkin–Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 or a Unity 400 spectrometer in $CDCl_3$. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hertz. A Horizon™ Flash Chromatograph from BioTage Inc. was used for flash column chromatography. HPLC was performed on a Shimadzu LC-10AT instrument with a C18 Varian Dynamax column (5 μm, 250 × 10 mm) and a phenyl Varian Dynamax column (5 μm, 250 × 10 mm). Finnigan LTQ LC/MS with a C18 Hypersil column (5 μm, 100 × 2.1 mm) was used for the analysis of compound **2** [20% MeCN, 20% MeCN, 100% MeCN, 100% MeCN (1% formic acid); 0 min, 5 min, 20 min, 30 min; Flow rate: 0.2 mL/min; t_R: 23 min].

3.2. In vitro phosphatase assays

Bioassay-directed fractionation was conducted with an epitope-tagged (histidine$_6$) catalytic domain of human recombinant Cdc25B, which contained amino acids 275–539 of the full-length protein and has been previously described.[21] The histidine$_6$-tagged catalytic domain and full-length Cdc25B were isolated and purified from *E. coli* with Ni-NTA resin as described previously.[21] Human recombinant VHR and PTP1B

phosphatases were purchased from BIOMOL (Plymouth Meeting, PA). Activities of all phosphatases were measured using the substrate *O*-methyl fluorescein phosphate (Sigma, St. Louis, MO) in a 96-well microtiter plate assay based on previously described methods.[21,22] The final incubation mixtures (25 μL) were prepared with a Biomek 2000 laboratory automation workstation (Beckman Coulter, Inc., Fullerton, CA). Fluorescence emission from the product was measured after a 20 or 60 min incubation period at ambient temperature with a multiwell plate reader (PerSeptive Biosystems Cytofluor II; Framingham, MA; excitation filter, 485 nm/bandwidth 20 nm; emission filter, 530 nm/bandwidth 25 nm). For the initial bioassay-directed fractionation, samples were evaluated at one concentration (0.2–1.0 μg/mL) and subsequent fractionations were examined with a minimum of six concentrations to determine the concentration required to inhibit enzyme activity by 50% (IC_{50}). To test for sensitivity to redox regulation, in some studies with full length Cdc25B, we adjusted the final concentration of DTT in the enzyme buffer, which contained 30 mM Tris (pH 8.5), 75 mM NaCl, 1 mM EDTA, and 0.033% bovine serum albumin, from the standard of 1 mM to a range from 0 to 100 mM. No significant difference was seen in inhibition with different DTT concentrations.

3.3. Marine sponge material

The sponge species (Family Thorectidae, Genus *Thorectandra*) was collected by the Coral Reef Research Foundation in May 1998 in Papua New Guinea at a depth of 30 M. The voucher specimen is deposited at the Smithsonian Institution under the code 0CDN5764, and a photograph of the sponge collection is available as supporting data.

3.4. Extract preparation

The frozen sponge was pulverized at the National Cancer Institute in dry ice by use of a worm-fed grinder (hamburger mill), the powder produced was allowed to stand at −30 °C until the CO_2 sublimed, and the mass was then extracted at 4 °C with de-ionized water (1 L) by stirring (30 rpm) for 30 min. The mixture was centrifuged at rt and the supernatant lyophilized to give the aqueous extract. The insoluble portion from the centrifugation was lyophilized and then statically extracted overnight at rt with 1 L of a 1:1 ratio of MeOH–CH_2Cl_2. The organic phase was filtered off, the pellet washed with a 10 vol % of fresh MeOH, and the combined organic phases reduced to dryness at <35 °C by rotary evaporation and then finally dried under high vacuum at rt to give the organic extract as a gum. An extract of this sponge was received from the National Cancer Institute as sample number C018781 (5.1 g).

3.5. Extraction and isolation

The crude extract (1.2 g; IC_{50} = 8 μg/mL) was suspended in aqueous MeOH (MeOH–H_2O, 9:1, 100 mL) and extracted with hexanes (3 × 100 mL). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 extract (542 mg) was found to be the most active with an IC_{50} value of 6 μg/mL. Ten fractions (I–X), of which fraction IV was found to be the most active, were collected from the CH_2Cl_2 extract by chromatography on a C18 column on a Biotage Horizon flash chromatograph using 85% MeOH–H_2O, followed by 100% MeOH. Compounds 16-oxo-luffariellolide (**1**, 12 mg, t_R 18 min), 16-hydroxy-luffariellolide (**2**, 5 mg, t_R 19 min), and luffariellolide (**4**, 20 mg, t_R 38 min) were obtained from fractions V, VI, and IX, respectively. Seven fractions (A–G) were collected from fraction IV with a Shimadzu preparative HPLC over C18 using 80% MeOH–H_2O, followed by 100% MeOH, and compound **5** was purified from fraction A (2.5 mg, t_R 15 min). A mixture of compounds **3** and **6** (3.0 mg, t_R 10 min) was obtained from the most active fraction (fraction D, IC_{50} 2.5 μg/mL) by elution with MeOH–H_2O (80:20) from a phenyl HPLC column. This mixture was further separated using silica gel TLC (CH_2Cl_2–MeOH, 100:15) to give **3** (1.4 mg; R_f = 0.4) and **6** (1.3 mg; R_f = 0.3).

3.5.1. 16-Oxoluffariellolide (1). Oil; $[\alpha]_D^{24}$ ±0 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ε) 251 (4.2) nm; IR (film) ν_{max} 3307, 2965, 2918, 2859, 1759, 1737, 1655, 1643, 1603, 1443, 1378, 1336, 1274, 1125, 948, 884; ^{1}H NMR and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* 401.2691 (calcd for $C_{25}H_{37}O_4$ 401.2692).

3.5.2. 16-Hydroxyluffariellolide (2). Oil; $[\alpha]_D^{24}$ +17 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 210, 260 (sh) nm; IR (film) ν_{max} 3307, 2918, 2859, 1735, 1646, 1447, 1124, 948, 858; ^{1}H NMR and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* 385.2747 (calcd for $C_{25}H_{37}O_3$ [M−OH] 385.2743); Finnigan LTQ LC/MS *m/z* 401 [M−H].

3.5.3. (2*E*,6*E*,10*E*)-3-formyl-7,11-dimethyl-13-(2,6,6-trimethylcyclohex-1-enyl)trideca-2,6,10-trienoic acid (3). Oil; UV (MeOH) λ_{max} (log ε) 210, 267 (sh) nm; IR (film) ν_{max} 2924, 2859, 2362, 2336, 1729, 1695, 1590, 1376, 1080; ^{1}H NMR and ^{13}C NMR data, see Table 1; (−)-HRESIMS *m/z* 385.2717 (calcd for $C_{25}H_{37}O_3$ 385.2743).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.04.070.

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