

Cytotoxic Metabolites from an Indonesian Sponge *Lendenfeldia* sp.Jingqiu Dai,[†] Yang Liu,[†] Yu-Dong Zhou,* and Dale G. Nagle*

Department of Pharmacognosy and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848

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The lipid extract of an Indonesian *Lendenfeldia* sp. sponge inhibited hypoxia-induced hypoxia-inducible factor-1 (HIF-1) activation in T47D breast tumor cells. Chromatographic separation yielded the new substituted naphthalene dimer **1**, the new furanolidipid **2**, and three known homoscalarane sesterterpenes, **3–5**. Compounds **1** and **3–5** inhibited hypoxia-induced HIF-1 activation (IC₅₀ values: 0.64–6.9 μM), but also reduced the viability of T47D and MDA-MB-231 breast tumor cells. Compound **4** was the most potent and showed a unique tumor cell line selectivity in the NCI 60-cell line panel. The general cytotoxicity of these compounds precluded their further consideration as HIF-1 inhibitors.

Marine natural products continue to be an invaluable source of new molecular-targeted antitumor agents.¹ An ongoing research program was initiated to discover potent and selective small-molecule inhibitors of hypoxia-mediated tumor cell adaptation, survival, and metastatic spread.² The primary molecular target for this drug discovery effort is the transcription factor hypoxia-inducible factor-1 (HIF-1), a heterodimer composed of the oxygen-regulated HIF-1α and the constitutively expressed HIF-1β subunits.³ Numerous studies strongly support HIF-1 as a valid molecular target for drug discovery that targets tumor hypoxia.⁴ Terrestrial and marine organisms have been shown to produce natural products that inhibit HIF-1.⁵ The NCI Open Repository of marine invertebrates and algae lipid extracts was examined for HIF-1 inhibitory activity using a T47D human breast carcinoma cell-based reporter assay.² The crude extract of the sponge *Lendenfeldia* sp. (Spongiidae) inhibited hypoxia-induced HIF-1 activation (99% inhibition at 5 μg mL⁻¹).

The extract (4 g) was purified by silica gel column chromatography and preparative TLC to yield two structurally unrelated new compounds (**1** and **2**) and three known homoscalarane sesterterpenes (**3–5**). Each of the *Lendenfeldia* compounds was identified by comparison of their spectroscopic data with those reported in the literature and by interpretation of ¹H NMR, ¹³C NMR, ¹H–¹H COSY, ¹H–¹³C HMQC, and ¹H–¹³C HMBC spectra.

Compound **1** was obtained as a colorless gum with the molecular formula C₂₂H₁₈O₆, as deduced from analysis of the HRESIMS data. The ¹H NMR spectra (Table 1) of **1** exhibited the presence of a methoxyl resonance at δ_H 4.02 ppm and four aromatic proton resonances (δ_H 7.39, 7.32, 7.16, and 6.70 ppm). While the HRESIMS suggested that the structure of **1** contains 22 carbons, the ¹³C NMR spectrum (Table 1) only exhibited 11 carbon resonances, thus indicating that the structure is a symmetrical dimer. The ¹H–¹H COSY and ¹H–¹³C HMQC spectra indicated that **1** contained two distinct aromatic ¹H–¹H spin systems: –CH(3)–CH(4)– and –CH(7)–CH(8)–. The ¹H–¹³C HMBC spectrum of **1** exhibited long-range correlations from C-2 to H-3, H-4, and C-2–OCH₃; from C-1 to H-3 and H-8; from C-5 to H-4 and H-7; from C-9 to H-4, H-7, and H-8; and from C-10 to H-3, H-4, and H-8. Therefore, the substitution pattern for each of the symmetrically substituted naphthalene ring systems was readily established. Compound **1** was optically active ([α]_D²⁵ +10.4). The CD spectrum displayed a positive split Cotton effect, indicating that **1** exhibits a right-handed helicity, signifying “S”-configuration. Thus, the structure of **1** was determined to be (*S*)-2,2'-dimethoxy-1,1'-binaphthyl-5,5',6,6'-tetraol.

* To whom correspondence should be addressed. (Y.-D.Z.) Tel: (662) 915-7026. Fax: (662) 915-6975. E-mail: ydzhou@olemiss.edu. (D.G.N.) Tel: (662) 915-7026. Fax: (662) 915-6975. E-mail: dnagle@olemiss.edu.

[†] These coauthors contributed equally to this work.

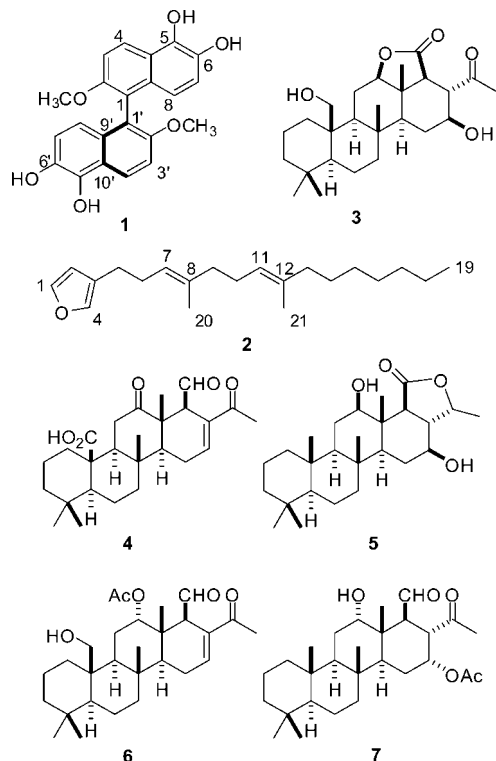
Table 1. ¹H (400 MHz) and ¹³C (100 MHz) NMR Data for **1** (CDCl₃)

position	δ _C	δ _H
1/1'	117.4	
2/2'	145.0	
3/3'	118.2	6.70 brs
4/4'	130.2	7.39 brs
5/5'	150.5	
6/6'	138.5	
7/7'	120.2	7.16 brs
8/8'	127.4	7.32 brs
9/9'	119.9	
10/10'	118.5	
11/11'	61.5	4.02 s

Compound **2** was isolated as a colorless oil. The HRESIMS indicated that the molecular formula of **2** is C₂₁H₃₄O. The ¹H NMR spectrum (Table 2) exhibited resonances typical of a β-substituted furan [δ 7.33 (1H, brs), 7.20 (1H, brs), 6.27 (1H, brs)]. The ¹³C NMR spectrum (Table 2) contained resonances for 21 carbons, and the ¹³C DEPT spectrum indicated the presence of three methyl, 10 methylene, five methane, and three quaternary carbon atoms. Analysis of the ¹H–¹H COSY and ¹H–¹³C HMQC spectra suggested that the structure of **2** contained four spin systems: –CH(1)–CH(2)–, –CH₂(5)–CH₂(6)–CH(7)–, –CH₂(9)–CH₂(10)–CH(11)–, and an unsaturated heptane chain –CH₂(13)–CH₂(14)–CH₂(15)–CH₂(16)–CH₂(17)–CH₂(18)–CH₃(19)–. The ¹H–¹H spin systems were connected through the observation of long-range ¹H–¹³C correlations in the HMBC spectrum from C-3 to H-1, H-2, H-4, H-5, and H-6; from C-8 to H-6, H-7, H-9, H-10, and H-20; and from C-12 to H-10, H-11, H-13, H-14, and H-21. Therefore, the structure was deduced to be that of a new furanolidipid.

The ¹H, ¹³C, and ¹³C DEPT NMR spectra of **3–5** were closely comparable with those of three previously reported *Lendenfeldia* homoscalarane sesterterpenes, namely, 16β,22-dihydroxy-24-methyl-24-oxoscalarane-25,12β-olactone (**3**),⁶ 24-methyl-12,24,25-trioxoscalar-16-en-22-oic acid (**4**),⁶ and 12,16-dihydroxy-24-methylscalarane-25,24-olide (**5**),⁷ respectively.

The effects of **1–5** on HIF-1 activity were examined initially in a cell-based reporter assay.² The level of HIF-1 activation was measured using the T47D breast tumor cell line that was transiently transfected with the pHRE-TK-Luc reporter as described previously.² Compound **2** was essentially inactive and showed no significant inhibition at 10 μM. Compounds **1** and **3–5** significantly inhibited both hypoxia-induced (IC₅₀ values 4.3, 6.9, 0.64, and 3.5 μM, respectively) and iron chelator (1,10-phenanthroline)-induced HIF-1 activation in T47D breast tumor cells (Supporting Information). Within the 16 h time frame of the HIF-1 assay, compounds **1** and **4** inhibited HIF-1 activation at concentrations that were



significantly lower than the concentrations at which these substances suppressed tumor cell viability. However, the marginal selectivity of these compounds for inhibition of HIF-1 activation (pHRE-TK-Luc) did not translate into a similar level of selectivity relative to their effect on cell viability after 48 h. In standard 48 h Neutral Red viability assays, only incrementally higher concentrations of **1** and **3–5** were required to reduce cell viability in T47D (hypoxic IC₅₀ values: 8.3, 7.2, 1.9, and 5.3 μ M) and MDA-MB-231 (hypoxic IC₅₀ values: 7.0, 8.3, 1.5, and 5.4 μ M) breast tumor cells. Compounds **1** and **3–5** produced similar effects on T47D and MDA-MB-231 breast tumor cells under normoxic conditions (Supporting Information).

Homoscalarane sesterterpenes have been reported to be cytotoxic to various tumor cell lines.^{8,9} However, most of these compounds have not been comprehensively examined for both cytostatic and cytotoxic activities. Therefore, compounds **3–5** were examined in the broad range of tumor cell lines represented by the National Cancer Institute's 60-cell line panel.¹⁰ Both **3** and **5** were found to inhibit tumor cell growth with very little specificity for individual tumor cell lines (average GI₅₀ values 20.4 and 19 μ M, respectively; Supporting Information). However, compound **4** was significantly more potent (mean GI₅₀ 1.17 μ M; mean LC₅₀ 11.2 μ M) than either of the other two related homoscalaranes (Supporting Information). Compound **4** also produced a more distinct cell line selectivity pattern of tumor cell growth suppression (GI₅₀ range 0.26 to 3.55 μ M) and cytotoxicity (LC₅₀ range 3.4 to >100 μ M). Two other structurally analogous homoscalaranes, PHC-4 (**6**)⁹ and scalarherbacin A (**7**),¹¹ produce equally potent tumor cell responses compared with **4** when evaluated in the NCI 60-cell line panel (Supporting Information). This would tend to suggest that the free C-25 aldehyde moiety found in the structure **4** is essential for more potent antitumor effects and that lactonization of this functional group (as in **3** and **5**) results in a marked decrease in potency. These compounds will not be considered for further development as HIF-1 inhibitors due to their considerable cytotoxicity and apparent lack of HIF-1 selectivity.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a RUDLPH Research Autopol IV/589-546 digital polarimeter. The IR spectrum was obtained using an AATI Mattson Genesis Series FTIR

Table 2. ¹H (400 MHz) and ¹³C (100 MHz) NMR Data for **2** (CDCl₃)

position	δ_C	δ_H (J in Hz)	position	δ_C	δ_H
1	142.6	7.33 brs	12	135.6	
2	111.0	6.27 brs	13	39.7	2.04 m
3	124.9		14	24.2	1.30 m
4	138.7	7.20 s	15	29.7	1.28 m
5	25.0	2.45 t (7.2)	16	28.4	1.28 m
6	28.2	2.38 t (7.2)	17	31.9	1.28 m
7	123.8	5.13 m	18	22.7	1.30 m
8	134.5		19	14.1	0.90 brs
9	39.2	2.02 q (7.2)	20	15.8	1.61 s
10	26.5	2.10 m	21	15.9	1.61 s
11	124.6	5.19 m			

spectrometer. UV spectra were recorded on a Varian 50 Bio spectrophotometer. The CD data were recorded in CH₂Cl₂ on a JASCO-J710 spectrometer. The NMR spectra were recorded in CDCl₃ on a Bruker AMX-NMR spectrometer operating at either 400 MHz for ¹H or 100 MHz for ¹³C. The NMR spectra were recorded running gradients and using residual solvent peaks (δ 7.27 for ¹H and δ 77.0 for ¹³C) as internal references. HRESIMS were measured using a Bruker Daltonic micro TOF with electrospray ionization. Silica gel (200–400 mesh) was used for column chromatography. TLC was run on Merck Si₆₀F₂₅₄ or Si₆₀RP₁₈F₂₅₄ plates and visualized under UV at 254 nm or by heating after spraying with a 1% anisaldehyde solution in acetic acid–H₂SO₄ (50:1).

Sponge Material. The sponge material was obtained from the National Cancer Institute's Open Repository Program. Gray-colored *Lendenfeldia* sp. (Spongiidae) was collected at 2 m depth on May 22, 1993 (sample C011337), from a sea grass bed in Indonesia by the Australian Institute of Marine Science and identified by Dr. Michelle Kelly (National Institute of Water and Atmospheric Research Limited, Auckland, New Zealand). A voucher specimen was placed on file with the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC. The sponge material was frozen at –20 °C and ground in a meat grinder.

Extraction and Isolation. Ground sponge material was extracted with H₂O. The residual sample was then lyophilized and extracted with CH₂Cl₂–MeOH (1:1), solvents were removed under vacuum, and the crude extract was stored at –20 °C in the NCI repository at the Frederick Cancer Research and Development Center (Frederick, MD). The crude *Lendenfeldia* sp. extract (4 g) was subjected to a process of bioassay-guided fractionation. Four fractions were produced by silica gel column chromatography (40 g), using a step gradient of hexanes–EtOAc–MeOH (85:15:0, 50:50:0, 0:100:0, 0:0:100). The active fraction that eluted with 50% hexanes in EtOAc (35.0 mg) was separated by column chromatography over 400 mg of silica gel with hexanes–EtOAc (2:1) to give **1** (10.0 mg, 0.25% yield), **2** (11.0 mg, 0.27% yield), and **5** (6.0 mg, 0.15% yield). The active fraction that eluted with 100% EtOAc (45.1 mg) was separated by column chromatography over 450 mg of silica gel with hexanes–CH₂Cl₂–EtOAc (1:1:2) to give **3** (7.0 mg, 0.17% yield) and **4** (10.0 mg, 0.25% yield).

(S)-2,2'-Dimethoxy-1,1'-binaphthyl-5,5',6,6'-tetraol (1): colorless gum; [α]_D²⁵ +10 (*c* 0.03, CH₂Cl₂); IR (KBr) ν_{\max} 3379, 2934, 2841, 1720, 1408, 1253 cm⁻¹; UV (CH₂Cl₂) λ_{\max} (log ϵ) 230 (4.30), 285 (3.46); CD $\Delta\epsilon_{\max}$ [λ (nm)] +7.15 \times 10³ (237.4), –4.16 \times 10³ (222.7); ¹H and ¹³C NMR data in Table 1; HRESIMS *m/z* 378.1105 (calcd for C₂₂H₁₈O₆, 378.1103).

Furanolipid (2) {IUPAC: 3-[(3E,7E)-4,8-Dimethylpentadeca-3,7-dienyl]furan}: colorless oil; IR (KBr) ν_{\max} 3125, 2915, 2853 cm⁻¹; UV (CH₃OH) λ_{\max} (log ϵ) 209 (4.48); ¹H and ¹³C NMR data in Table 2; HRESIMS *m/z* 302.2609 (calcd for C₂₁H₃₄O 302.2610).

16 β ,22-Dihydroxy-24-methyl-24-oxoscalaran-25,12 β -olactone (3): ACS Registry number 81575-75-9; IUPAC 2S,3S,3aS,5aR,6aS,6bR,10aS,12aR,12bS,12cS)-3-acetyloctadecahydro-2-hydroxy-6b-(hydroxymethyl)-10,10,12a,12c-tetramethyl-4H-chryseno[12,1-bc]furan-4-one.

24-Methyl-12,24,25-trioxoscalar-16-en-22-oic acid; PHC-1 (4): ACS Registry number 81575-76-0; IUPAC (4aR,4bS,6aS,7S,10aS,10bS,12aS)-8-acetyl-7-formyl-1,3,4,4b,5,6,6a,7,10,10a,10b,11,12,12a-tetradecahydro-1,1,6a,10b-tetramethyl-6-oxo-4a(2H)-chrysenecarboxylic acid.

12,16-Dihydroxy-24-methylscalaran-25,24-olide (5): ACS Registry number 135048-59-8; IUPAC (5 α ,12 β ,16 β ,17 α ,17a β)-17a,20-lactone-12,16,20-trihydroxy-4,4,8-trimethyl-D(17a)-homopregnane-17a-carboxylic acid.

Cell-Based Luciferase Assay. Human breast carcinoma-derived T47D and MDA-MB-231 cells (ATCC) were maintained in DMEM/F12 media with glutamine (Mediatech) supplemented with 10% FCS (v/v, Hyclone), 50 U mL⁻¹ penicillin G sodium, and 50 μ g mL⁻¹ streptomycin (Biowhittaker) in a humidified environment under 5% CO₂/95% air at 37 °C. Transfection, compound treatment, exposure to hypoxic conditions and a hypoxia mimetic (10 μ M 1,10-phenanthroline), and measurement of luciferase activity were performed as previously described.² The test compounds were prepared as 4 mM stock solutions in DMSO and stored at -20 °C. The IC₅₀ values were determined using GraphPad Prism 4 software from experiments performed in triplicate. Compounds were tested at half-log concentrations, and the standard deviation values for over 95% of the data points are less than 15%.

Neutral Red Assay for Cell Proliferation/Viability. Detailed description of the assay procedure was as previous published.¹² The following formula was used to calculate % inhibition of cell proliferation/viability: % inhibition = 1-OD₅₄₀(treated)/OD₅₄₀(control).

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Supporting Information Available: Tables with IC₅₀ values (μ M) of **1** and **3–5** on HIF-1 activation, 16 h cell proliferation/viability in T47D breast tumor cells, and 48 h cell proliferation/viability in T47D and MDA-MB-231 breast tumor cells. Copies of the NCI/DTP 60-cell line GI₅₀, TGI, and LC₅₀ mean bar graphs for compounds **3–7**. The material is available free of charge via the Internet at <http://pubs.acs.org>.

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