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Cytotoxic Metabolites from an Indonesian Sponge *Lendenfeldia* sp

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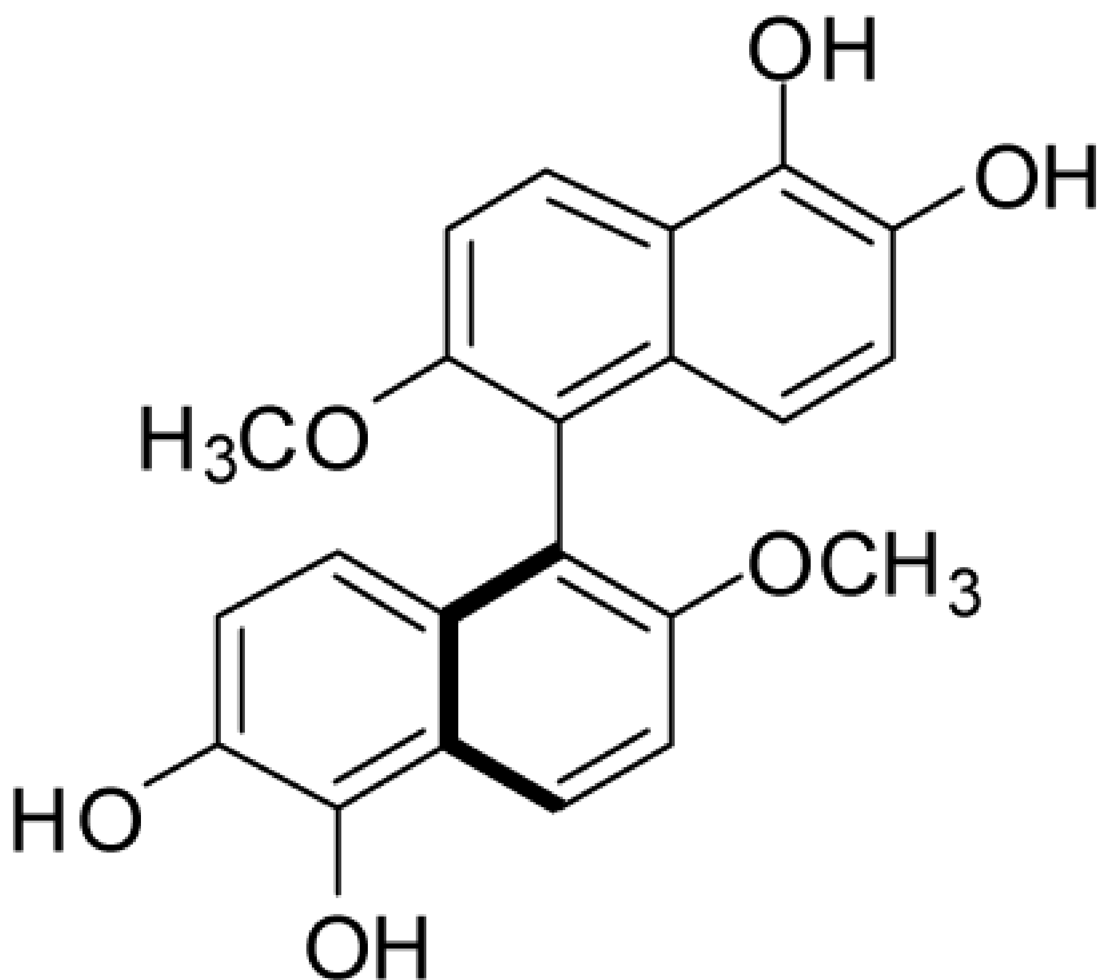
Abstract



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 furanolipid **2**, and three known

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homoscalarane sestertepenes **3 - 5**. Compounds **1** and **3 - 5** inhibited hypoxia-induced HIF-1 activation (IC_{50} 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.

Compound **1**

Hypoxia-induced HIF-1 inhibition: IC_{50} 4.6 μM

T47D Breast tumor cell proliferation/viability (16 h): IC_{50} 19 μM

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 were identified by comparison of their spectroscopic data with those reported in the literature and by interpretation of ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC spectra.

Compound **1** was obtained as colorless gum with the molecular formula $C_{22}H_{18}O_6$, as deduced from analysis of the HRESIMS data. The ^{1}H NMR spectra (Table 1) of **1** exhibited the presence of a methoxyl resonance 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 ^{13}C NMR spectrum (Table 1) only exhibited eleven carbon resonances, thus indicating that the structure is a symmetrical dimer. The ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC spectra indicated that **1** contained two distinct aromatic ^{1}H-^{1}H spin systems:-CH(3)-CH(4)- and -CH(7)-CH(8)-. The ^{1}H-^{13}C HMBC spectrum of **1** exhibited long-range correlations from C-2 to H-3, H-4, C-2-OC$\underline{H}_3$; from C-1 to H-3, H-8; from C-5 to H-4, H-7; from C-9 to H-4, H-7, 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 ($[\alpha]^{25}_D$ +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.

Compound **2** was isolated as colorless oil. The HRESIMS indicated that the molecular formula of **2** is $C_{21}H_{34}O$. The ^{1}H 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 ^{13}C NMR spectrum (Table 2) contained resonances for 21 carbons, and the ^{13}C DEPT spectrum indicated the presence of three methyl, ten methylene, five methane, and three quaternary carbon atoms. Analysis of the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC spectra suggested that the structure of **2** contained four spin systems: -CH(1)-CH(2)-, -CH_2(5)-CH_2(6)-CH(7)-, -CH_2(9)-CH_2(10)-CH (11)-, and an unsaturated heptane chain -CH_2(13)-CH_2(14)-CH_2(15)-CH_2(16)-CH_2(17)-

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CH_2(18)-CH_3(19)-. The ^{1}H-^{1}H spin systems were connected through the observation of long-range ^{1}H-^{13}C correlations in the HMBC spectrum from C-3 to H-1, H-2, H-4, H-5, H-6; from C-8 to H-6, H-7, H-9, H-10, H-20; and from C-12 to H-10, H-11, H-13, H-14, H-21. Therefore, the structure was deduced to be that of a new furanolipid.

The ^{1}H, ^{13}C and ^{13}C 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-oxoscalaran-25,12β-olactone (**3**),[6] 24-methyl-12,24,25-trioxoscalar-16-en-22-oic acid (**4**),[6] and 12,16-dihydroxy-24-methylscalaran-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 (IC_{50} values 4.3, 6.9, 0.64, 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 IC_{50} values: 8.3, 7.2, 1.9, and 5.3 μM) and MDA -MB-231 (hypoxic IC_{50} values: 7.0, 8.3, 1.5, and 5.4 μM) 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 GI_{50} values 20.4 and 19 μM, respectively; Supporting Information). However, compound **4** was significantly more potent (mean GI_{50} 1.17 μM; mean LC_{50} 11.2 μM) 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 (GI_{50} range 0.26 to 3.55 μM) and cytotoxicity (LC_{50} range 3.4 to >100 μM). 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 spectrometer. UV spectra were recorded on a Varian 50 Bio spectrophotometer. The CD data were recorded in CH_2Cl_2 on a JASCO-J710 spectrometer. The NMR spectra were recorded in

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$CDCl_3$ on a Bruker AMX-NMR spectrometer operating at either 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. The NMR spectra were recorded running gradients and using residual solvent peaks (δ 7.27 for ^{1}H) and (δ 77.0 for ^{13}C) 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 $Si_{60}F_{254}$ or $Si_{60}RP_{18}F_{254}$ plates and visualized under UV at 254 nm or by heating after spraying with a 1% anisaldehyde solution in acetic acid-H_2SO_4 (50:1).

Sponge Material

The sponge material was obtained from the National Cancer Institute's Open Repository Program. Grey 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, D.C. The sponge material was frozen at -20 °C and ground in a meat grinder.

Extraction and Isolation

Ground sponge material was extracted with H_2O. The residual sample was then lyophilized and extracted with CH_2Cl_2-MeOH (1:1), solvents were removed under vacuum, and the crude extract stored at -20 °C in the NCI repository at the Frederick Cancer Research and Development Center (Frederick, Maryland). 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 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 silica gel with hexanes-CH_2Cl_2-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; $[\alpha]^{25}_D$ +10 (*c* 0.03, CH_2Cl_2); IR (KBr) ν_{max} 3379, 2934, 2841, 1720, 1408, 1253 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε) 230 (4.30), 285 (3.46); CD $\Delta\varepsilon_{max}$ [λ(nm)] +7.15 × 10^3 (237.4), -4.16 × 10^3 (222.7); ^{1}H and ^{13}C NMR data in Table 1; HRESIMS *m/z* 378.1105 (calcd for $C_{22}H_{18}O_6$ 378.1103).

Furanolipid (2) {IUPAC: 3-[(3*E*,7*E*)-4,8-Dimethylpentadeca-3,7-dienyl]-furan}

Colorless oil; IR (KBr) ν_{max} 3125, 2915, 2853 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 209 (4.48); ^{1}H and ^{13}C NMR data in Table 2; HRESIMS: *m/z* 302.2609 (calcd for $C_{21}H_{34}O$ 302.2610).

16β,22-Dihydroxy-24-methyl-24-oxoscalaran-25,12β-olactone (3)

ACS Registry number: 81575-75-9; IUPAC: (2*S*,3*S*,3a*S*,5a*R*,6a*S*,6b*R*,10a*S*,12a*R*,12b*S*, 12c*S*)-3-acetyloctadecahydro-2-hydroxy-6b-(hydrox ymethyl)-10,10,12a,12c-tetramethyl-4*H*-chryseno[12,1-*bc*]furan-4-one.

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24-Methyl-12,24,25-trioxoscalar-16-en-22-oic acid; PHC-1 (4)

ACS Registry number: 81575-76-0; IUPAC: (4a*R*,4b*S*,6a*S*,7*S*,10a*S*,10b*S*,12a*S*)-8-Acetyl-7-formyl-1,3,4,4b,5,6,6a,7,10,10a,10b,11,12,12a-t etradecahydro-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: (5α,12β,16β,17α,17aβ)-17a,20-lactone-12,16,20-trihydroxy-4,4,8-trimethyl-D(17a)-homopre gnane-17a-carboxylic acid.

Cell-Based Luciferase Assay

Cultured T47D and MDA-MB-231 cells (ATCC) were maintained in DMEM/F12 media with glutamine (Mediatech) supplemented with 10% FCS (v/v, Hyclone), and 50 U mL^{-1} penicillin G sodium and 50 μg mL^{-1} streptomycin (Biowhittaker) in a humidified environment under 5% CO_2/95% air at 37 °C. Transfection, compound treatment, exposure to hypoxic conditions and a hypoxia mimetic (10 μM 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 IC_{50} 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-OD_{540}(treated)/OD_{540}(control).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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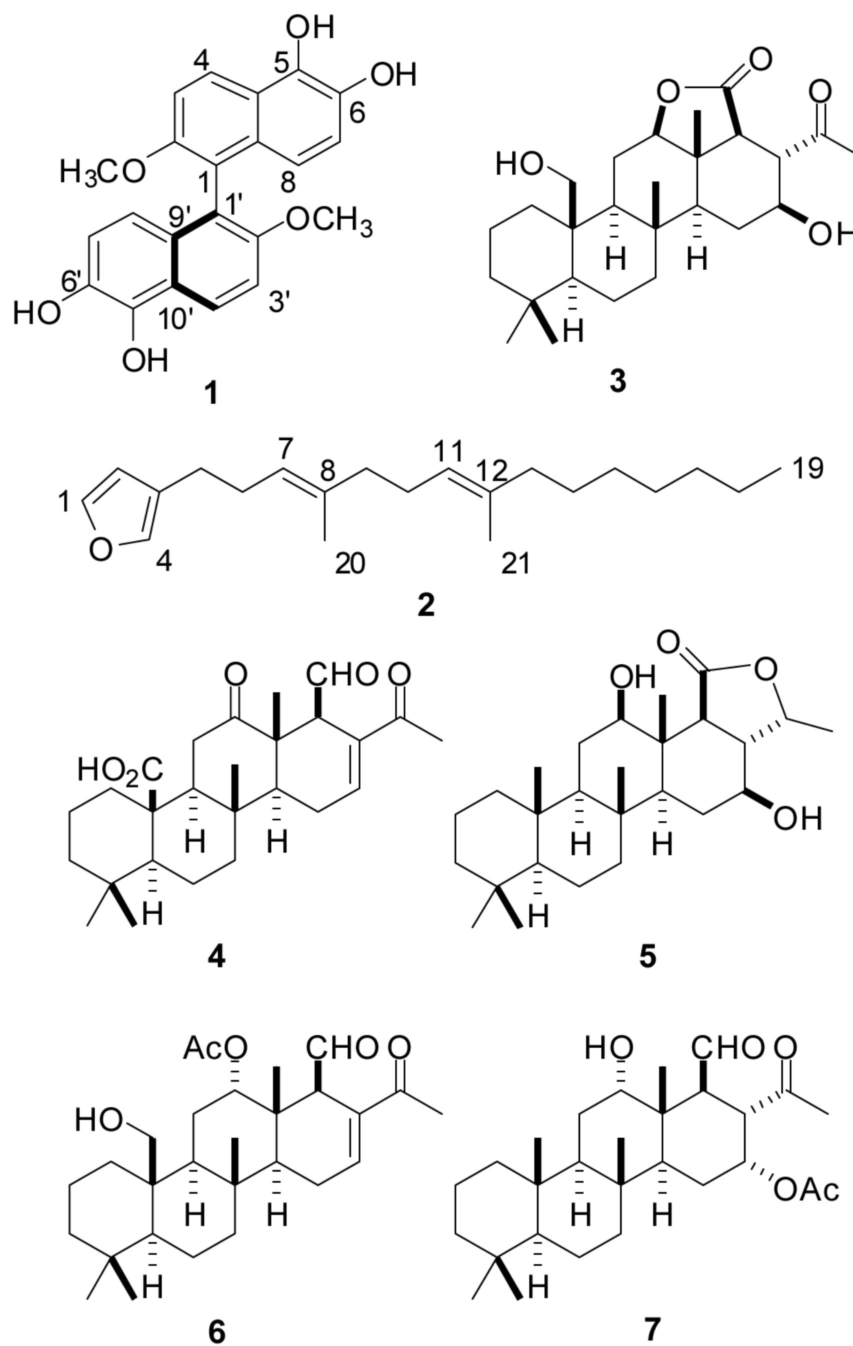

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Table 1

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for **1** ($CDCl_3$)

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

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Table 2

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for **2** ($CDCl_3$)

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			