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Ganodone, a Bioactive Benzofuran from the Fruiting Bodies of *Ganoderma tsugae*

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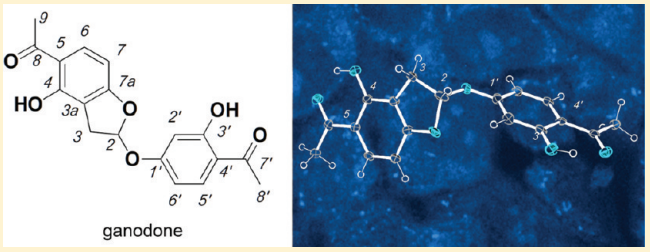
Supporting Information

ABSTRACT: Extracts of *Ganoderma tsugae*, also known as the Hemlock varnish shelf mushroom, and related Reishi mushrooms are well documented in traditional Chinese medicine. Several *Ganoderma* sp. are currently cultivated for use in coffee, teas, and dietary supplements. We now report on the isolation and characterization of an unprecedented benzofuran, ganodone (**1**), from the fruiting bodies of mature growth *G. tsugae*. This discovery provides a key next step in evaluating the active components in their associated herbal supplements.



For centuries, mushroom teas have been an integral facet of traditional Chinese medicine.[1−3] One mushroom in particular, *Ganoderma lucidum*, known as Ling Chih in China and Reishi in Japan, has been used extensively to treat a variety of conditions including arthritis, cancer, hepatitis, and insomnia.[4−6] It is cultivated as a commercial crop.[7−9] A related species, *G. tsugae*, is also believed to hold medicinal properties. Its extracts have been suggested to trigger multiple biological responses including the induction of gene expression,[8] the regulation of growth factor expression,[10] anti-inflammatory responses,[4,7,11,12] antioxidant activity,[13,14] and antitumor activity.[15,16] While *G. tsungae* mushroom extracts are documented historically, a comprehensive characterization of their biological compounds has yet to be completed. To date, a series of isolation efforts have led to the characterization of polysaccharides[17−21] and terpenoids[22−24] from extracts of *G. tsugae* and related species. We now report on the isolation and structure elucidation of a unique benzofuran, ganodone (**1**). Benzofuran-containing natural products have been isolated from a variety of mushrooms including ganofuran B from *G. lucidum*.[25] Other examples include auxofuran,[26,27] laetrobin,[28] kynapcin-24,[29] suillusin,[30] and vialinin B.[31]

Multiple specimens of *G. tsugae*, XRI3214−XRI3242 (Table S1, Supporting Information), were collected at different stages of growth on Eastern Hemlock trees, *Tsuga canadensis*, in the Delaware Creek basin, a tributary of Lake Erie, near Angola, New York (near latitude 42.63965, longitude −79.058803). Each specimen was extracted in EtOH at 4 °C for 24 h. The EtOH fractions were filtered, dried via rotary evaporation, and defatted by partitioning between CH_3CN and hexanes. Samples of each CH_3CN fraction were then screened for their ability to inhibit tumor cell progression by applying the MTT assay to HCT-116 cells (Table S1, Supporting Information).[32,33] Extracts from the older specimens (Figure 1a) displayed increased activity when compared to those from the younger budding specimens (Figure 1b,c). An increased activity was also observed in parasitic specimens when compared to those growing on dead wood (saprobic growth). LC-MS screening indicated an increased concentration of a compound with $[M + H]^+$ at m/z 329 within the more active, older parasitic extracts (such as in XRI3227, Figure 2a) and the lack of this mass signature in the less active, younger, and saprobic specimens (such as in XRI3125, Figure 2b).[34]

On the basis of this evidence, we applied 3 g aliquots of extract XRI3227 or XRI3228 to a 60 mm i.d. dry column vacuum chromatography (DCVC)[35] column charged with 500 g of Merck grade 15111 silica gel 60 (15−40 μm). Six fractions were collected as described in the Experimental Section. LC/MS analyses indicated that the 1:10 MeOH/EtOAc and 1:5 MeOH/EtOAc fractions contained a compound with the $[M + H]^+$ at m/z 329. These fractions were then combined and then subjected to either flash chromatography or HPLC purification to deliver an off-white solid, compound **1**. Small crystals with a melting point of 229−231 °C were obtained by perfusion of MeOH into a saturated solution of **1** in CH_2Cl_2. High-resolution mass analysis returned an exact mass for $[M + H]^+$ at m/z 329.1026, suggesting a molecular formula of $C_{18}H_{16}O_6$.[36] Analysis of this material using HPLC and LC-MS indicated greater than 99% purity, as depicted in Figure 2c. Furthermore, we also observed only a single peak when applying samples of **1** to a Chirobiotic T chiral column (Figure 2d), suggesting that **1** was a single enantiomer. This was further supported by the observation of optical activity with samples of **1** displaying an $[\alpha]^{20}_D$ of 19.2 ± 0.4 (*c* 0.41, acetone).

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Figure 1. *Ganoderma tsugae*: (a) specimen XRI3227, a parasitic and mature specimen; (b) XRI3215, a saprobic bud; and (c) specimen XRI3221, a saprobic and grown specimen. Ganodone (**1**) was found in extracts of specimens XRI3227 (7.7 mg/kg) and XRI3228 (6.0 mg/kg) but not in extracts of specimens XRI3215 and XRI3221. All images were taken in the field prior to harvesting.

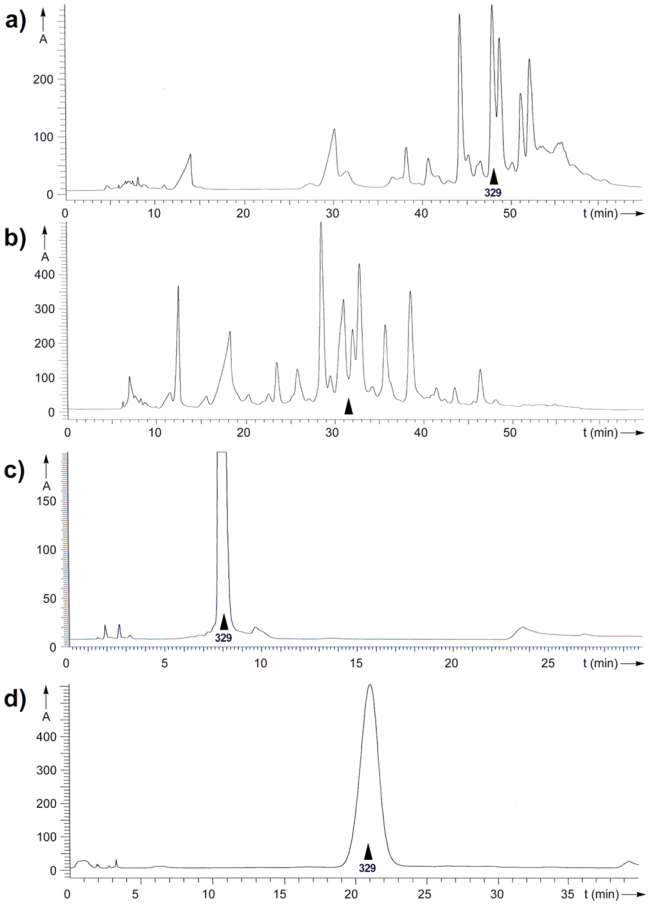

Figure 2. Purification of ganodone (**1**). (a) An LC/MS chromatogram collected with absorption at 254 nm denoting the position of **1** (329 *m*/*z*) in the CH_3CN defatted extract XRI3227. (b) LC/MS chromatogram depicting the metabolites present in the CH_3CN defatted extract XRI3125. The retention time for **1** was determined by spiking as noted by the arrow. (c) LC/MS chromatogram collected on an Ultrasphere C19 column confirming the purity of crystalline ganodone (**1**). (d) LC/MS trace depicting samples of crystalline **1** run on a Chirobiotic T column denoting the presence of a single peak. The peaks displaying a $[M + H]^+$ at 329 *m*/*z* are denoted on each chromatogram.

The ^1H NMR spectrum of **1** in $CDCl_3$ contained 16 protons comprised of two phenolic protons (δ_H 13−14 ppm), five aryl protons (δ_H 6−8 ppm), two methyl groups (δ_H 1−2 ppm), and three aliphatic protons (δ_H 2−7 ppm). The ^{13}C NMR spectrum contained a total of 18 carbons including two ketonic carbonyls (δ_C 200−205 ppm), 12 aryl carbons (δ_C 100−170 ppm), one heteroatom-substituted aliphatic carbon (δ_C 104 ppm), and three additional aliphatic carbons (δ_C 25−35 ppm). The number of carbons and protons observed (Table 1) was in agreement with the molecular formula, $C_{18}H_{16}O_6$, obtained by HRMS analysis.

Analysis of gCOSY and TOCSY spectra (copies provided in the Supporting Information) identified three structural units. The first was an ABX system containing two geminally coupled protons H-3α and H-3β that were vicinal to methine proton H-2 ($J_{2-3\alpha}$ = 8.4 Hz and $J_{2-3\beta}$ = 2.5 Hz in $CDCl_3$). The large geminal coupling between protons H-3α and H-3β ($J_{3\alpha\text{-}3\beta}$ = 21.2 Hz in $CDCl_3$) was suggestive of a five-membered ring (typically observed with J = 18−21 Hz).[37] The second unit was a trisubstituted aryl moiety containing three protons, H-2′, H-5′, and H-6′ (see data in Table 1). The coupling constant analysis indicated that aryl proton H-6′ was *ortho* to H-5′ ($J_{5'\text{-}6'}$ = 10.9 Hz in $CDCl_3$)[38] and *meta* to H-2′ ($J_{2'\text{-}6'}$ = 3.1 Hz in $CDCl_3$). The third unit was also an aryl moiety that contained two protons, H-6 and H-7. The presence of a large coupling constant (J_{6-7} = 10.8 Hz) indicated that proton H-6 was *ortho* to proton H-7. On the basis of this evidence, this unit contained a 1,2,3,4-tetrasubstitution.

On the basis of chemical shift analysis, protons H-5′ and H-7 were in an electron-deficient environment, while H-6′ and H-6 were in an electron-rich environment. The IR spectrum suggested that the electron-withdrawing environment arose from acetyl groups (ν_{max} 1608). This conclusion was further supported by the presence of two ketonic carbonyls in the ^{13}C NMR spectrum (δ_C 203.4, 203.1), along with the respective methyl groups in both the ^1H NMR and ^{13}C NMR spectra (δ_H 1.41 s and δ_C 26.6, 26.8). On the basis of the molecular formula as well as evidence from IR (ν_{max} 3402) and ^1H NMR (δ_H 14.11 s, 13.97 s) spectra, the electron-donating environment arose from phenols. From these data, we determined that the first two units comprised a 4-hydroxy-5-aceyl-2,3-dihydrobenzofuran. The remaining aryl unit was a 2,4-dihydroxyacetophenone. A summary of the ^1H NMR and ^{13}C NMR data is provided in Table 1, and the key gCOSY cross-peaks are provided in Figure 3a.

NOESY spectra were collected on multiple instruments (copies provided in the Supporting Information), and the salient nuclear Overhauser effects (NOE) are reported in Figure 3b. They include a cross-peak between the methine proton H-2 of the dihydrobenzofuran and protons H-2′ and H-6′ on the 2,4-dihydroxyacetophenone (circled in Figure 3c). This evidence suggested that the 2,4-dihydroxyacetophenone was attached via the phenolic oxygen *para* to its acetyl group (at carbon C-1′; see structure in Figure 3) to carbon C-2 of the dihydrobenzofuran (see Figure 3b).

HSQC, HMQC, and HMBC spectra were collected using a X-Sens NMR cold probe (copies provided in the Supporting

Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Ganodone (1)

position	δ_C, mult.	δ_H, mult. (J in Hz)	COSY[a]	NOESY	HMBC[b]
2	104.4[c1], CH	6.27 m	3α,3β[e]	3α,3β,2′	3[e],3a,7a,1′
3α	33.7, CH_2	2.58 dd (21.2, 8.4)	2	2	4,5,7a
3β		2.40 dd (21.1, 2.5)	2	2	5,7,7a
3a	111.5, C				
4	160.4, C				
4-OH		14.11 s[c2]			3a,4,5,7a
5	115.8, C				
6	132.7[d3], CH	7.74 d (10.8)	7	7,9	3a[e],4,5[e],8
7	102.6, CH	6.27 d (10.8)	6	6	3a,5,7a
7a	164.6, C				
8	203.4[c3], C				
9	26.6[d2], CH_3	1.41 s[d3]		6	5,6,8
1′	162.6, C				
2′	104.3[c1], CH	6.57 d (3.0)	6′	2	1′,3′,4′,6′
3′	165.0, C				
3′-OH		13.97 s[c2]			1′,2′,3′,4′
4′	115.5, C				
5′	133.3[d1], CH	7.78 d (11.1)	6′	6′,8′	2′ [e],3′,4′[e],7′
6′	108.7, CH	6.40 dd (10.9, 3.1)	2′,5′	5′	1′,2′,4′
7′	203.1[c3], C				
8′	26.8[d2], CH_3	1.41 s[d3]		6′	4′,5′,7′

[a] An additional cross-peak between protons H-2′ and H-5′ was observed in the TOCSY spectrum. [b] HMBC data, optimized for 8 Hz, are given from δ_H to δ_C. [c1−c3] These peaks were distinguished using data from the NOESY or HMBC spectra or a combination thereof. [d1−d3] The 2D data set failed to provided a clear identification of these pairs of peaks. [e] This cross-peak was weak.

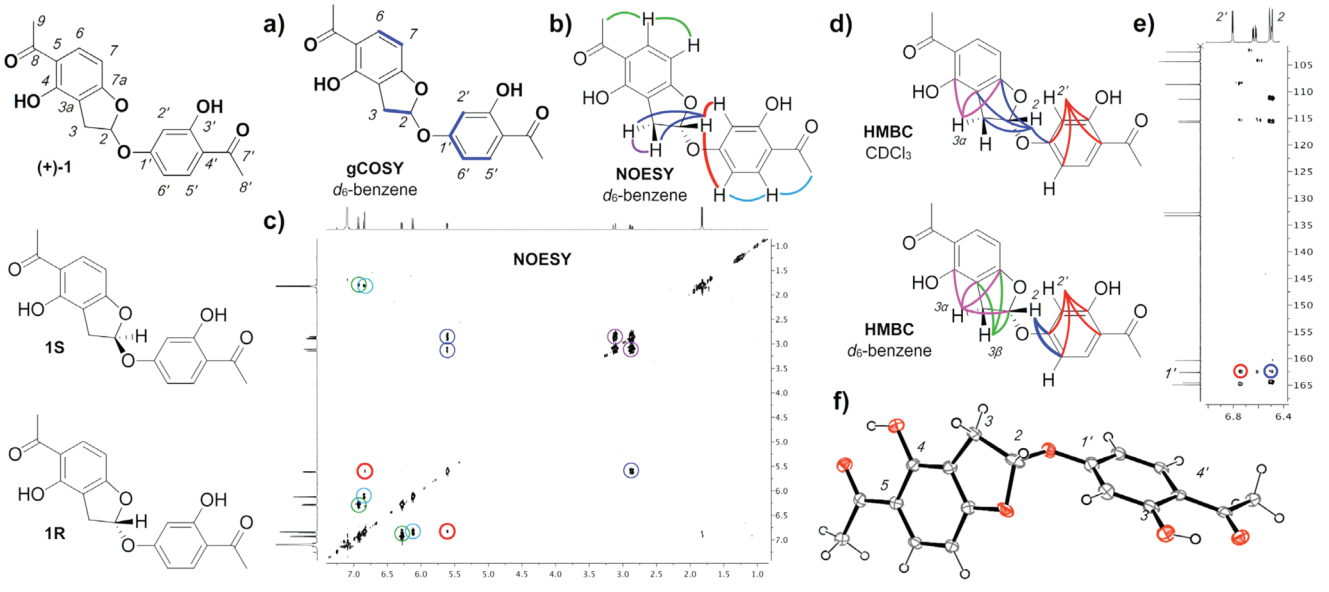

Figure 3. Salient data supporting the elucidated structure of ganodone (**1**) including (a) gCOSY data (shown in blue); (b) key NOESY interactions in d_6-benzene; (c) an expansion of the NOESY interactions of **1** in d_6-benzene (cross-peaks are color coded according to the interactions shown in item b); (d) key HMBC data of **1** in d_6-benzene or $CDCl_3$; (e) an expansion of the HMBC spectrum of **1** in $CDCl_3$ depicting the key interactions that link proton H-2 to carbon C-1′ (red) and proton H-2′ to carbon C-1′ (blue); and (f) X-ray crystal structure of **1** with ellipsoids depicted at 50%.

Information). HSQC and HMQC spectra were used to assign the protons and carbons (Table 1). The HMBC data provided further support for the assignment of **1**. The methylene protons H-3α and H-3β were particularly useful in this analysis (Figure 3d). The presence of a cross-peak between methine proton H-2 and carbon C-1′ (circled in Figure 3e) supported our proposed linkage between the dihydrobenzofuran and dihydroxyacetophenone units (see structure in Figure 3). While indicative, the fact that the key protons H-2 and H-7 appeared at overlapping chemical shifts created a degree of uncertainty. This problem was solved after screening multiple solvents (12 screened), with d_6-benzene providing the optimal resolution of H-2. While only

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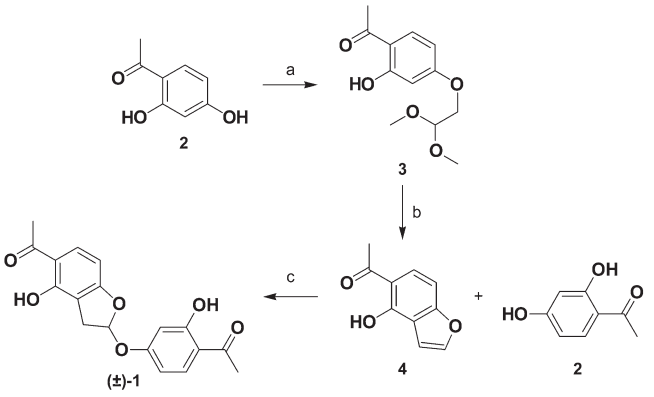
Table 2. NMR Spectroscopic Data (500 MHz, C_6D_6) for Ganodone (1)

position	δ_C, mult.	δ_H, mult. (J in Hz)	COSY	NOESY	HMBC[a]
2	104.6, CH	5.68 dd (2.1,6.8)	3α,3β[c]	3α,3β,2′,6′	1′,6′
3α	34.0, CH_2 0.62, CH_2	2.94 dd (6.7, 16.9)	2	2	2,3a,4,5,7a
3β		3.18 dd (2.0, 16.9)	2	2	2,3a,7a
3a	112.0, C				
4	161.2, C				
4-OH		13.38 s[d1]			3a,4,5,7a
5	116.1, C				
6	133.6[b], CH	6.91 d (8.6)	7	7,9	3a,4,5,7a,8
7	102.5, CH	6.19 d (8.6)	6	6	3a,4,5,7a
7a	165.0, C				
8	203.4[d2], C				
9	26.3[d4], CH_3	1.90 s[d3]		6	5,6,8
1′	163.1, C				
2′	104.5, CH	6.90 d (2.6)	6′	2	1′,3′,4′,6′
3′	166.0, C				
3′-OH		13.34 s[d1]			1′,2′,3′,4′
4′	115.8, C				
5′	133.0,[b] CH	7.00 d (8.9)	6′	6′,8′	1′,2′,3′,4′,7′
6′	109.0, CH	6.35 dd (2.5,8.9)	2′,5′	2,5′	1′,2′ [c],3′,4′
7′	203.1[d2], C				
8′	26.1[d4], CH_3	1.88 s[d3]		6′	4′,5′,7′

[a] HMBC data, optimized for 8 Hz, are given from δ_H to δ_C. [b] These peaks were identified by the cross-peaks between 5′ and 8′, and 6 and 9, in the NOESY spectrum. [c] This cross-peak was weak. [d1−d4] These sets of peaks were differentiated using data from the NOESY and/or HMBC spectra.

Scheme 1. Synthesis of Racemic Ganodone (±)-(1)[a]



[a] Reagents and conditions: (a) $BrCH_2CH(OEt)_2$, K_2CO_3, DMF, 89%; (b) Amberlyst 15, toluene, reflux over a Dean-Stark trap, 6 h, 32%; (c) TMSOTf, CH_2Cl_2, −78 to −40 °C, 2 h, 42%.

modestly soluble in benzene (1 mg/mL), access to the high carbon sensitivity of the X-Sens probe allowed effective collection of the ^{13}C NMR, HSQC, and HMBC spectra, as summarized in Table 2. This data also supported the assigned structure (see Figure 3).

Small diffraction-quality crystals were obtained by perfusing toluene into a saturated solution of **1** in 3:1 CH_2Cl_2/EtOH. X-ray crystallographic measurements confirmed the structural assignment (Figure 3f). While the data suggested an *R*-configuration at C-2, the lack of resolution at this center prevented the accurate calculation of the Flack *x* parameter, and hence, the absolute configuration could not be determined. Attempts at applying Mosher ester analysis or circular dichroism (CD) spectroscopy also failed. The latter was complicated due to the lack of standards of pure **1R** or **1S** isomers.[39] Therefore, we were not able to determine the absolute configuration of **1**. As noted, all evidence including chiral LC-MS analysis as well as the presence of a strong optical rotation suggest that **1** was obtained as a single enantiomer (either **1R** or **1S** in Figure 3).

We further confirmed the structural assignment through chemical synthesis. As illustrated in Scheme 1, samples of racemic (±)-**1** were prepared in three steps from 2,4-dihydroxyacetophenone (**2**). This began by preparation of benzofuran **4** by alkyation of **2** to deliver acetal **3** followed by treatment of **3** with Amberlyst 15 resin using the methods of Goel.[40] A second molecule of acetophenone **2** was then coupled by treating a mixture of **2** and **4** with catalytic amounts of TMSOTf in CH_2Cl_2 at low temperature. Overall, this procedure delivered racemic (±)-**1** in 12% yield from commercially available 2,4-dihydroxyacetophenone (**2**).

Activity analyses were conducted on samples of natural **1**. Using the MTT assay,[32,33] we determined that **1** displayed potent cytostatic activity in HCT-116, HeLa, and Neuro2a cell lines with respective IC_{50} values of 0.22 ± 0.01, 0.49 ± 0.03, and 0.081 ± 0.019 μM after treatment with a single dose for 24 h. As modest fluorescence was obtained from **1** with maxima at λ_{ex} = 316 nm, λ_{em} = 420 nm in phosphate-buffered saline pH 7.2 containing 1% DMSO, we were able to examine the uptake and subcellular localization of **1** in tumor cells using confocal fluorescence microscopy equipped with a blue channel for image collection (λ_{ex} = 377 ± 50 nm, λ_{em} = 448 ± 20 nm). Cells were treated either with a single concentration of **1** applied over the entire imaging period or with **1** for 1 h followed by washing the cells and reincubating in fresh media. In both experiments, compound **1**

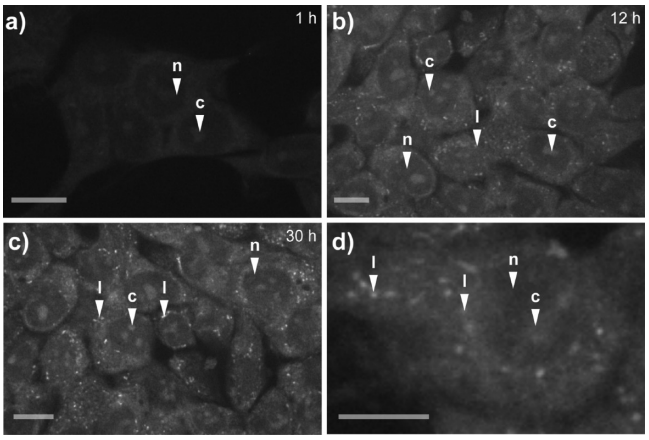


Figure 4. Uptake and subcellular localization of ganodone (**1**) in HCT-116 colon cancer cells. (a) Confocal fluorescent microscopic images depicting the uptake, and HCT-116 cells treated with media containing 1 μM **1** for 1 h indicate a modest uptake. (b) Cells treated under the same conditions for 12 h display specular localization (l) in the cytosolic space and near the nucleus (n) as well as localization within the chromatin (c). (c) Cells treated with 1 μM **1** for 3 h then washed and incubated for an additional 30 h indicate comparable subcellular localization. (d) Close-up of the subcellular localization observed in cells treated with 1 μM **1** for 3 h then washed and incubated for an additional 30 h.

appeared throughout the cell and concentrated over 12−24 h within the specular regions in the cytosol and around the nuclear membrane (Figure 4). Counterstaining against lysosomes, peroxosomes, and mitocondria failed to identify these regions (not shown), indicating that further studies are required before conclusions can be made as to the origin of this subcellular localization.[41]

While sufficient sources of the natural material can be obtained for further preclinical studies,[42] the development of a synthetic route to single isomers **1R** or **1S** would be useful in providing material for structure activity relationship (SAR) studies and related biological investigations. The observed antiproliferative activity and effective subcellular localization suggest that compounds related to **1** would be worthy of evaluation as potential medicinal leads. This could include the use of **1** as a template for synthetic libraries.[43−47] Our studies are now focused on further evaluation of **1** with the goal of defining its targets and mode of regulating tumor cell proliferation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer-241 MC polarimeter. UV−vis spectra were collected on a PTI QuantaMaster UV−vis steady-state spectrofluorometer with a 0.1 cm quartz cell at 23 °C. FT-IR spectra were collected using a thin film on NaCl plates using a Perkin-Elmer Spectrum One FT-IR spectrometer. HRMS spectra were collected on a ThermoFinnigan MAT900XL-MS. NMR data were recorded on a Varian VX500 equipped with a XSens cold probe or a JEOL ECA500 spectrometer. Samples were dissolved in $CDCl_3$ or in d_6-benzene with the assistance of an ultrasonic bath, and data collected in either 3 mm or 5 mm NMR tubes. Chemical shifts were calculated relative to the $CDCl_3$ solvent peak (δ_H at 7.24 and δ_C at 77.23) or d_6-benzene (δ_H at 7.16 and δ_C at 128.38). HMBC spectra were collected with $J_{1,3}$ = 8 Hz. NMR spectra were processed with MestreNova version 6.0.2-5475. Solvents were purchased from VWR Scientific or Fisher Scientific. Unless otherwise noted, solvent ratios are noted in (v/v). HPLC purification and LC/MS analyses were conducted from multiple instruments, columns, and solvent mixtures. Exemplary conditions include the use of a Waters model 600E liquid chromatograph with a Waters Wisp 712 autoinjector, a Waters 486 tunable absorbance detector, and a St. Johns 2001A Fluoro-Tec filter fluorometer. HPLC analysis was conducted on a 4.5 × 250 mm Beckman Ultrasphere C18 (5 μm) ion pairing or Waters Symmetry C8 (5 μm) column at 23 °C with a flow rate of 1.2 mL/min. Exemplary runs include isocratic runs with 20% aqueous MeOH (5:1 MeOH/H_2O), 30% aqueous MeOH, 45% aqueous MeOH, as well a 35 min binary linear gradient from H_2O to 5% aqueous MeOH. Detection was conducted with diode array detection monitored at 254 and 272 nm. The masses of the identified peaks were determined by collection of the eluted fractions followed by MS analysis or by direct LC/MS analysis. Chiral HPLC was conducted using similar elution conditions on a 3.9 × 150 mm Chirobiotic T (4.6 × 250 mm, 5 μm, Supelco 12024AST) chiral HPLC column operated at 23 °C with a 1.0 mL/min flow rate, and the column was run under conditions that resolved peptide and other small molecule standards.[48]

Extract Preparation. Fruiting bodies were collected from multiple samples of *G. tsugae* at different stages of growth (Table S1, Supporting Information). Voucher samples were deposited at the Xenobe Research Institute in San Diego, CA. After collection in the field, each specimen was shipped to San Diego within 24 h of harvest and stored at −20 °C upon arrival. Extracts were prepared using an identical procedure for each specimen. Fungal fruit bodies were broken into 100−300 cm^2 squares soaked in absolute EtOH (2 L per kg of wet mass) in Erlenmeyer flasks for 24 h at 4 °C. The flasks were periodically shaken. The EtOH was filtered through a coarse sintered glass funnel and concentrated by rotary evaporation to deliver a crude extract. The crude extract was then partitioned between 1:2 (v/v) mixture of CH_3CN/hexanes. The CH_3CN layer was collected and dried via rotary evaporation.

Isolation of Ganodone (1). LC-MS analysis was used to guide the isolation of compound **1** with a $[M + H]^+$ m/z of 329. A combination of DCVC and flash chromatography was used to obtain pure samples of **1**. An exemplary procedure was as follows: a 3 g aliquot of extract XRI3227 was dissolved in CH_2Cl_2, loaded on a 20 cm high by 20 mm i.d. DCVC column loaded with 500 g of Merck grade 15111 silica gel 60 (15−40 μm), and eluted by successive passing of 1 L aliquots of 2:1 hexanes/EtOAc, 1:1 hexanes/EtOAc, EtOAc, 1:10 MeOH/EtOAc, 1:5 MeOH/EtOAc, and 1:2:XXX MeOH/EtOAc/Et_3N. Each fraction was subjected to analytical LC/MS analysis. The 329 m/z peak appeared in 1:10 MeOH/EtOAc and 1:5 MeOH/EtOAc fractions. These fractions were combined and applied to flash chromatography using a 20 cm high by 20 cm i.d. column loaded with EM Sciences Geduran silica gel 60 and eluting with a gradient from 1:1 hexanes/EtOAc to 1:2 EtOAc/MeOH. This process provided 32.5 mg of an off-white solid that delivered 21.2 mg of pure **1** after crystallization from vapor diffusion of MeOH into a saturated solution of **1** in CH_2Cl_2. Alternatively, preparative HPLC purification can be used on the crude DCVC fractions with a gradient of H_2O to 10% aqueous MeOH (1:10 H_2O/MeOH). Application of this procedure to the XRI3228 extract delivered 19.2 mg of pure **1** after crystallization.

X-ray Crystallography. Crystals were grown by vapor diffusion of toluene into a saturated solution of **1** in 3:1 CH_2Cl_2/EtOH. A crystal of **1** was mounted on a glass fiber, and X-ray data were collected with a Bruker AXS SMART APEX diffractometer using Cu (Kα) radiation at 100 K with the SMART suite of programs (SMART, version 5.628, 2001, Bruker AXS). Data were processed and corrected for Lorentz and polarization effects with SAINT (SAINT+, version 6.22a, 2001, Bruker AXS Inc., Madison, WI, USA) and for absorption effect with SADABS (SADABS, version 2.10, 2001, G. W. Sheldrick, University of Goettingen, Germany). Structural solution and refinement were carried out with the SHELXTL suite of programs (SHELXTL, version 6.14, 2000, Bruker AXS). The structure was solved by direct methods, followed by

difference maps for the light, non-hydrogen atoms. All non-hydrogen atoms were given anisotropic displacement parameters in the final mode. All H-atoms were put at calculated positions; 3836 reflections were collected and 934 were unique (R_{int} = 0.0392). Direct methods were used to solve the structure, and all non-hydrogen atoms were refined anisotropically. All H atoms were placed in idealized locations. For $C_{18}H_{16}O_6$, orthorhombic, $P2(1)2(1)2$, a = 5.4674(9) Å, b = 29.890(4) Å, c = 4.4627(9) Å, V = 729.3(2) Å^3, Z = 4, D_x = 1.500 Mg/m^3, R_1 = 0.0568, wR_2 = 0.1650 based on $2\sigma(I)$ data. An ORTEP depiction of the structure is provided in Figure 3f. The coordinates have been uploaded to the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk/).

Racemic Synthesis of (±)-Ganodone (1). TMSOTf (19.0 μL, 0.1 mmol) was added to a stirred solution of benzofuran **4**[40] (176.2 mg, 1.0 mmol) and dihydroxyacetophenone **2** (152.1 mg, 1.0 mmol) dissolved in CH_2Cl_2 (10 mL) containing powdered 4 Å molecular sieves (100 mg) at −78 °C. The solution was kept at this temperature for 3 h and then warmed to −40 °C over 2 h. The reaction was terminated by the addition of saturated $NaHCO_3$ (30 mL). After warming to rt, the reaction mixture was extracted with CH_2Cl_2 (3 × 40 mL), washed with brine (20 mL), and dried with Na_2SO_4. Pure racemic (±)-**1** (137.6 mg, 42%) was obtained after flash chromatography (2:1 hexanes/EtOAc to 2:1 EtOAc/MeOH) followed by crystallization by the perfusion of MeOH to (±)-**1** saturated in CH_2Cl_2. The product matched the spectral properties of the isolated samples of **1** with the exception of the optical rotation and melting point, which for (±)-**1** were mp 216−218 °C (corrected against camphor at 179−180 °C) and $[\alpha]^{20}_D$ of 0.06 ± 0.4 (*c* 0.49, acetone). Copies of ^{1}H and ^{13}C spectra from synthetic (±)-**1** are provided in the Supporting Information.

Bioactivity Assays. Briefly, cells were incubated with media containing **1** for 24 h. The studies were first conducted with 10-fold dilutions from 100 μM to 0.1 nM. Cytotoxicity was evaluated using the MTT procedure.[32,33] The inflection point was noted, and the assays were then repeated within the range detected (i.e., a compound that delivered IC_{50} values between 1 and 0.1 μM was then rescreened at 10 intervals within this range). All assays were conducted in repetitions, and the data were reported as an average over five analyses. IC_{50} values were determined using etoposide, IC_{50} = 3.2 ± 0.5 μM, as a positive control.

Uptake and Localization in HCT-116 Cells. HCT-116 cells (ATCC CCL-247) were cultured in Dulbecco's modification of Eagle's medium with 4.5 g L^{-1} glucose, 4.5 g L^{-1} L-glutamine, and 5% heat-inactivated fetal calf serum in glass-bottom dishes. Fluorescent images were collected on a Leica DMI6000 inverted confocal microscope with a Yokogawa spinning disk confocal head, Hamamatsu Orca ER high-resolution B&W cooled CCD camera with 6.45 μm/pixel at 1×, Plan Apochromat 40×/1.25 na and 63×/1.4 na objective, and a Melles Griot Argon/Krypton 100 mW air-cooled laser for excitation at 488, 568, and 647 nm. Localization was confirmed by counterstaining cells exposed to **1** to either Syto-60 (nucleus), LysoTracker Red DND-99 (lysosomes), BODIPY TR glibenclamide (endoplasmic reticulum), or MitoTracker Red 580 (mitochondria) for 20 min, washing the cells three times with media, and collecting images in multiple channels.

Ganodone (1): off-white orthorhombic crystals (MeOH diffusion into CH_2Cl_2), mp 229−231 °C (corrected against camphor at 179−180 °C); $[\alpha]^{20}_D$ 19.2 ± 0.4 (*c* 0.41, acetone); UV−vis (MeOH) λ_{max} (log ε) 236 (3.2), 316 (1.6); IR (thin film) ν_{max} 3402 (br), 1608 (br), 1582, 1501, 1379, 1332, 1286, 1269, 1198, 1172 (w), 1160 (w), 1147 (w), 1112 (w), 1065, 1040, 956, 902, 834, 803 cm^{-1}; NMR data in $CDCl_3$ and C_6D_6, Tables 1 and 2, respectively; HRESIMS $[M + H]^+$ m/z 329.1026 (calcd for $C_{18}H_{17}O_6$ 329.0947).

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H, ^{13}C, and selected 2D NMR spectra of ganodone (**1**) and synthetic (±)-**1** are available free of charge via the Internet at http://pubs.acs.org.

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