A Pentahalogenated Monoterpene from the Red Alga *Portieria hornemannii* Produces a Novel Cytotoxicity Profile against a Diverse Panel of Human Tumor Cell Lines†

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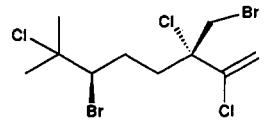
A polyhalogenated acyclic monoterpene, 6(*R*)-bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene (1) was obtained as a major component of the organic extract of the red alga *Portieria hornemannii*. X-ray diffraction analysis provided the complete structure, including correct placement of the different halogen atoms and determination of the absolute stereochemistry. Detailed NMR analyses provided complete ^1H and ^{13}C assignments. Compound 1 exhibited highly differential cytotoxicity against the U.S. National Cancer Institute's new in vitro human tumor cell line screening panel; brain tumor, renal, and colon tumor cell lines were most sensitive to 1, while leukemia and melanoma lines were relatively less sensitive. A second collection of *P. hornemanni* yielded the novel, monocyclic 2, considerably less cytotoxic and devoid of differential activity. On the basis of its unprecedented cytotoxicity profile in the NCI primary screen, compound 1 has been selected by the NCI Decision Network Committee for preclinical drug development.

Introduction

Since the mid-1970s Rhodophyta (red algae) have been known to produce halogenated monoterpenes.[1] Although scores of acyclic, monocyclic, and bicyclic halogenated monoterpenes have been identified,[2] this class of compounds has been confined to the genera *Plocamium* and *Chondrococcus*. The structure elucidation of these compounds has not been a simple task. The relatively volatile monoterpenes tend to decompose under EI-MS conditions and acquisition of molecular weight and formula information has often been difficult. Correct placement of chlorine and bromine substituents has not proven to be straightforward, as NMR chemical shift arguments are clouded by the cumulative effects of multiple substituents on the C_{10} skeleton. We present here the X-ray crystal structure, absolute stereochemistry, complete ^1H and ^{13}C NMR assignments, and novel spectrum of differential cytotoxicity of 6(*R*)-bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene (1), from the red alga *Portieria hornemannii*.



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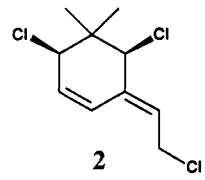

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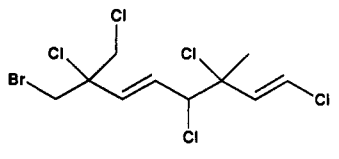

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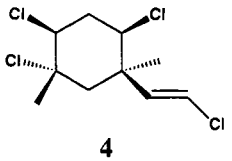

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Chemistry

Organic extracts of *P. hornemannii* (Lyngbye) P. C. Silva (*Chondrococcus hornemannii*), which were initially selected for study on the basis of the finding of activity in the National Cancer Institute's in vitro anti-HIV screening assay,[3,4] were partitioned between CH_2Cl_2 and CH_3OH–H_2O (1:19). The antiviral CH_2Cl_2 solubles were permeated through Sephadex LH-20. The seventh of seven fractions was crystallized from methanol to give 1, which was inactive in the anti-HIV screen. Preliminary ^1H and ^{13}C NMR analyses suggested a monoterpene. While EI-MS failed to provide a discernible molecular ion or M – HX fragment ion, CI-MS did reveal a weak pseudomolecular ion cluster beginning at m/z 416 ($M + NH_4^+$), which corresponded to the molecular formula $C_{10}H_{15}Br_2Cl_3$; this was confirmed by high-resolution EI-MS. The structure was solved by X-ray diffraction analysis (see Figure 1). To our knowledge, this is only the third X-ray crystal structure of a naturally occurring halogenated monoterpene.[5,6]

A compound proposed to have the same structure as 1 was reported previously by Burreson et al.[7] as an unre-

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† This paper is offered in memory of Professor Gordon Pagenkopf, a valued colleague whose battle with a brain tumor ended February 16, 1987.

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Table I. ^1H and ^{13}C NMR Assignments for Compounds 1 and 2[a]

carbon/ hydrogen no.	1		2	
	^{13}C[b]	^1H[c]	^{13}C[b]	^1H[c]
1	118.5	5.61 (d, 2.4) 5.84 (d, 2.4)	38.5	4.21 (dd, 11.7, 8.7) 4.13 (dd, 11.7, 7.7)
2	139.7		125.9	6.07 (ddd, 8.7, 7.7, 1)
3	73.8		134.5	
4	37.8	2.53 (ddd, 13.2, 10.2, 1.9) 2.14 (t, 10.2)	122.3	6.44 (ddd, 10.3, 2, 1)
5	30.1	2.48 (ddd, 13.2, 11.5, 1.5) 1.97 (dddd, 11.5, 11.2, 10.2, 1.9)	130.9	5.86 (dt, 10.3, 2)
6	64.6	4.02 (dd, 11.2, 1.5)	65.3	4.51 (br)
7	71.6		42.3	
8	27.1	1.66 (s)	28.1	4.40 (br)
9	38.6	3.83 (d, 10.7) 3.77 (d, 10.7)	26.6	1.23 (s)
10	33.1	1.77 (s)	26.6	0.98 (s)

[a] Recorded in $CDCl_3$ on a Varian VXR-500 spectrometer. [b] 125 MHz, δ. [c] 500 MHz, δ (multiplicity, J in Hz).

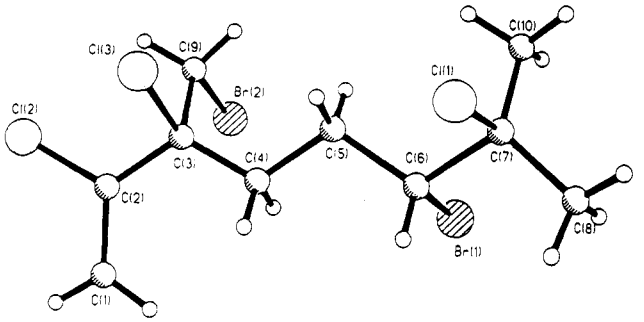

Figure 1. A computer-generated perspective drawing of the final X-ray model of compound 1. The absolute configuration was determined from anomalous scattering effects.

solved component in a mixture of monoterpenes from *C. hornemannii*; however, the material was only partially characterized. Here, in addition to our X-ray analysis, we have used ^1H–^1H COSY, HMQC, and HMBC experiments to assign definitively all the resonances in the ^1H and ^{13}C NMR spectra of 1 (see Table I). Given the renewed interest in halogenated monoterpenes[8-10] and the recent disclosure of insecticidal activity in this class of compounds,[10b,11] this fully elucidated structure may prove useful in resolving or precluding the difficulties encountered earlier[2,12] in assigning the location of halogens in these molecules.

Although compound 1 did not exhibit any HIV-inhibitory activity, the anti-HIV activity of the source extract was further tracked, via bioassay-guided chromatography and dereplication analysis, to traces of aplysiatoxin and debromoaplysiatoxin, presumably derived from a cyanobacterial contaminant or epiphyte in the algal collection.[13] Aplysiatoxin and debromoaplysiatoxin have been previously identified as the anti-HIV constituents of *Lyngbya majuscula* in our laboratory.[14]

A second collection of *P. hornemannii* was made from a different location, Central Visayas, in the Philippines. Analysis of the organic extracts revealed the absence of 1; instead, a new compound was isolated as the principal monoterpene in this collection. The structure 2 was assigned as a result of mass spectral ($C_{10}H_{13}Cl_3$ by HR-EI-MS), UV (λ_{max} 242 nm, ϵ = 3340), and NMR analyses. The ^{13}C-NMR spectra disclosed, in addition to the four conjugated sp^2 carbons suggested by the UV absorption, two methyls, a quaternary carbon, and three carbons bearing halogens (a methylene and two methines). The coupling relationships were readily discerned from ^1H–^1H COSY experiments, and the assembly of the various parts followed from the HMBC data. The relative stereochemistry was assigned from NOE experiments relationships of H-5 to H-4 and H-6, H-4 to H-1, H-8 to H-2; the absolute configuration was not determined.

Antitumor Screening Results and Discussion

While 1 was inactive in the anti-HIV assay, it appeared quite highly cytotoxic against the uninfected human lymphoblastoid control cells used in the AIDS-antiviral screen. This observation prompted us to evaluate 1 in the NCI's human tumor, disease-oriented in vitro screen.[15-18] In-

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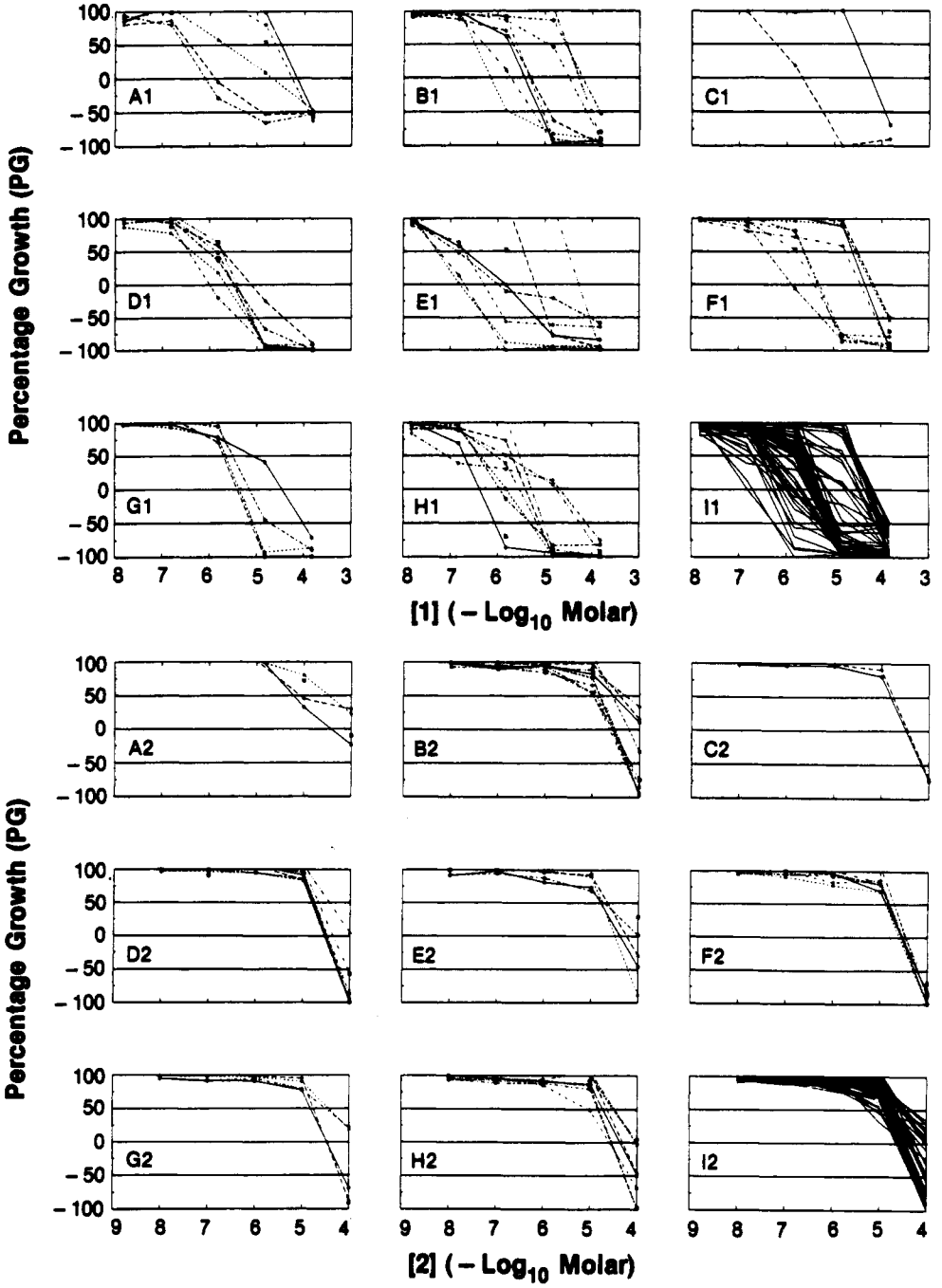

Figure 2. Dose–response curves from the testing of 1 (upper composite of nine graphs) and 2 (lower composite of nine graphs) against the cell line subpanels comprising the NCI's human tumor, disease-oriented in vitro screen. Individual cell line identifiers are omitted for clarity. Graphs A1 and A2 are from the leukemia/lymphoma subpanel, graphs B1 and B2 the non-small-cell lung cancer subpanel, graphs C1 and C2 the small-cell lung cancer subpanel, graphs D1 and D2 the colon cancer subpanel, graphs E1 and E2 the brain tumor subpanel, graphs F1 and F2 the melanoma subpanel, graphs G1 and G2 the ovarian cancer subpanel, graphs H1 and H2 the renal cancer subpanel, graphs 11 and 12 composites of all the respective subpanels together.

terestingly, there was an exceedingly broad range of in vitro sensitivities among the various types of human tumors to the cytotoxic effects of 1 (Figure 2; also see Experimental Section). For example, compared to some of the less sensitive melanoma and leukemia lines, several of the more sensitive brain, renal, and colon tumor cell lines were as much as 1000-fold or more sensitive to 1 at the GI_{50} response level and 100-fold at the LC_{50} level.

Compound 2 showed little differential cytotoxicity in the NCI screening panel, and it was at least 1 order of magnitude less potent at all three levels of analysis (GI_{50}, TGI, LC_{50}) than 1 (Figure 2; also see Experimental Section). Two related compounds, 3 and 4, from the NCI Repository also have been tested as part of the NCI's empirical screening program. The monocarbocyclic 4 gave a response similar to that of 2, showing some general cytotoxicity, but little differential among the various cell lines; the acyclic

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3 was somewhat more potent than 4 but exhibited only very modest differential activity (data not shown).

Other carbocyclic halomonoterpenes from Rhodophyta reportedly have shown general toxicity in brine shrimp assays[19] and in vitro inhibition of murine leukemia[20] or other[21] cell lines, but our results suggest further that certain human solid tumor cell lines may be particularly susceptible to the cytotoxic effects of some members of this class of compounds. The contrast between the acyclic and monocyclic compounds would suggest additionally that these compounds, particularly the acyclic ones, are not acting merely as electrophiles (alkylating agents); consistent with this view were results of computerized pattern-recognition studies, using the COMPARE algorithms,[17,18] which revealed no resemblance of the mean graph profiles (not shown) of 1 to known alkylating agents. Indeed, more extensive COMPARE pattern-recognition analyses likewise revealed little or no substantial resemblance of the screening profile of 1 to that of any known mechanistic or structural class of cytotoxic agent in the entire current (as of March 1, 1992) database.

Key features which make the screening profile of 1 unique can be appreciated visually in the concentration–response curves (Figure 2). While a multilog spread in relative cellular sensitivities is not uncommon at the GI_{50} (growth inhibitory) level of response, it is uncommon at the most demanding LC_{50} (cytotoxic or net cell killing) level of response. Moreover, the clustering of the relatively more sensitive lines among the renal, brain, colon, and non-small-cell lung cancer subpanels, along with the clustering of the relatively less sensitive lines among the leukemia and melanoma subpanels (see Experimental Section), is a highly unusual combination. A selection of those tumor cell lines showing the highest in vitro sensitivity to 1 will provide appropriate targets for detailed in vivo therapeutic investigations in appropriate xenograft models.

Further biological activity comparisons of 1 with compounds not yet tested in the NCI screen but reported in the literature to have antitumor or cytotoxic properties in other screening systems are problematic at the present time. Consistent or standardized reporting standards or formats for compounds tested in the new NCI screen need to be adopted by investigators more generally to facilitate such comparisons in the future. Herein, we offer two examples of complementary approaches to reporting comparative biological data derived from the new NCI in vitro primary screen.

Figure 2 exemplifies a composite which can be prepared directly from the laser-print graphical information typically included in the standard NCI screening data report package provided to all submitters of compounds to the NCI program. Extraneous, repetitive, or otherwise distracting information has been removed and the graphical scaling simplified to allow considerable photoreduction yet still vividly illustrate the contrasting concentration–response profiles.

While the reporting of screening results in simplified graphical formats may be very useful to illustrate, reinforce, contrast, or compare certain unique, rare, or otherwise highly unusual screening profiles such as those presented herein, we do not suggest that such graphical reporting necessarily should be adopted routinely for publication. Just as NMR, mass spectrometric, and other routine, documentary spectral data are reported typically in the chemical literature in a cryptic, alphanumerical rather than graphical format, a similar approach may be more efficient, and more acceptable to editors, for the routine reporting of data from the new NCI primary screen. An example of such an approach to an alphanumerical format is given for compound 1 and 2 in the Experimental Section. The information provided is sufficient to allow the reader to reconstruct a "mean graph" profile which, in turn, could be used for comparisons, either visually or by computer, with similarly reconstructed or otherwise reported screening profiles of known standards or other compounds of interest in the future.

In summary, we describe here the discovery of a naturally-occurring pentahalogenated monoterpene which produces a highly novel cytotoxicity profile against the diverse tumor types which comprise the NCI's primary screening panel. We have presented the biological activity information in a variety of formats both to illustrate the unusual spectrum of activity of this particular compound and to offer some complementary data-reporting alternatives that hopefully may contribute to the evolution of consistent reporting standards for investigators using the new NCI screen.

Compound 1 produces one of the most extreme examples of differential cytotoxicity observed thus far in the first 2 years of operation of the NCI screen. The compound clearly is not a general cell poison; its preferential cytotoxicity to cell lines derived from highly chemoresistant human tumors is intriguing. The NCI Decision Network Committee recently reached a similar consensus and selected compound 1 for drug development.[22] An immediate NCI priority is mass recollection (now ongoing) of *P. hornemannii* from the original collection site, as well as the investigation of chemical synthesis as an alternate source of the compound for preclinical development.

Experimental Section

Collection, Extraction, and Fractionation of *P. hornemannii*. Our original sample of *P. hornemannii* was collected by Ernani G. Menez near Chanaryan, Batan Island, in the Philippines in April 1986. A voucher specimen is on deposit at the Smithsonian Institution. The alga was frozen on collection, lyophilized, and extracted with CH_2Cl_2–MeOH (1:1), followed by a MeOH rinse. The crude organic extract (2.5 g) was partitioned between CH_2Cl_2 (100 mL) and H_2O–MeOH (19:1, 100 mL). The dichloromethane phase was reduced, in vacuo, and permeated through Sephadex LH-20 (column 2.5 × 150 cm) with CH_2Cl_2–MeOH (1:1). Seven fractions were obtained.

6(*R*)-Bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene (1). The seventh fraction from the above was crystallized from MeOH give 1: 55 mg; mp 49–50°; $[\alpha]_D$ +206° (*c* 1.08, CH_2Cl_2); CI-MS (NH_3): *m/z* 416/418/420/422/424 (MNH_4^+, 0.3/1.0/1.0/0.5/0.1), 336/338/340/342 (1.8/3.3/2.2/0.6), 300/302/304 (4.3/6.9/3.4), 247/249 (17/22), 203/205 (19/13), 167/169 (27/24), 96/66, 52 (100); HR-EI-MS *m/z* 401.8583 (calcd for $C_{10}H_{15}{}^{35}Cl^{37}Cl_2{}^{79}Br_2$, 401.8619), 399.8637 (calcd for $C_{10}H_{15}{}^{35}Cl_2{}^{79}Br_2$, 399.8679).

Single-Crystal X-ray Diffraction Analysis of 6(*R*)-Bromo-3(*S*)-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene (1). Compound 1 crystallized from *n*-pentane upon slow evaporation at −25 °C as clear thin plates, and a specimen with ap-

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(22) NCI Decision Network Committee, March 23, 1992, minutes. NCI Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

proximate dimensions 0.1 × 0.15 × 0.2 mm was selected for analysis. Preliminary X-ray photographs displayed orthorhombic symmetry. Accurate lattice constants of a = 6.115 (4) Å, b = 12.456 (6) Å, c = 19.188 (10) Å were determined from 30 diffractometer measured 2θ values. Systematic extinctions, optical activity, and crystal density were consistent with space group $P2_12_12_1$, with one molecule of composition $C_{10}H_{15}Br_2Cl_3$ forming the asymmetric unit. Additional crystallographic parameters were V = 1461.6 (14) Å^3, μ(Mo Kα) 6.02 mm^{-1}, and D_c = 1.824 g cm^{-3} for Z = 4. Intensity data were collected on a Nicolet (Siemens) $P2_1$ diffractometer with Mo Kα radiation (0.71073 Å) and a 2θ-θ scan technique. A total of 1157 Friedel unique data were collected, of which 686 (59%) with $|F_o| \geq 4\sigma|F_o|$ were considered observed after correction for Lorentz, polarization, and background effects. The structure was solved by direct methods and refined by full matrix, least-squares techniques.[23] The final refinements with anisotropic thermal parameters for all nonhydrogen atoms, riding isotropic hydrogens, and anomalous scattering corrections for bromine and chlorine converged smoothly to a final discrepancy index of 7.81% for the enantiomer shown (w_R = 6.72%). The other enantiomer converged to the significantly higher value of 8.51%. The absolute configuration also was ascertained by the η-test. The enantiomer shown refined to η = +1.1 (2).[23] A computer-generated perspective model of the final model is given in Figure 1; archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, Cambridge, U.K.

Second Collection of *P. hornemannii* and Isolation of 2. The subsequent collection of *P. hornemannii* (2.7 kg fresh weight) was made at Banilad, Bacong, Central Visayas, in the Philippines in April 1991, and kept frozen for transport and storage prior to extraction. The frozen alga was ground with dry ice in a hamburger grinder and extracted sequentially with MeOH–CH_2Cl_2 (3:1, then 2 × 1:1). The extracts were filtered and evaporated at 22 °C to an aqueous suspension, which was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were evaporated, again at 22 °C, to give 10.39 g of a dark green oil. A portion (1.7 g) of this extract was partitioned between CH_3OH–H_2O (19:1) and hexane. The hexane-soluble fraction (1.074 g) was permeated through Sephadex LH-20 (2.5 × 150 cm) with CH_2Cl_2–MeOH (1:1) to give seven fractions; the seventh fraction was essentially pure 1-[3-(1-chloro-2(*E*)-propenyl)]-2,4-dichloro-3,3-dimethylcyclohex-5-ene (**2**): 116 mg of colorless oil; [α] +71.3° (c 1.0, $CHCl_3$); UV λ_{max} (CH_2Cl_2) 242 nm (ϵ = 3340); HR-EI-MS m/z 240.0055 (calcd for $C_{10}H_{13}{}^{35}Cl_2{}^{37}Cl$, 240.0053), 238.0072 (calcd for $C_{10}H_{13}{}^{35}Cl_3$, 238.0083); LR-EM-MS m/z (relative intensity) 240/238 (M^+, 9/10), 205 (14), 203 (20), 189 (6), 187 (8), 169 (33), 167 (100), 153 (19), 133 (26), 131 (32), 117 (36), 115 (35).

Biological Testing Protocol: Data Display and Analysis. Pure compounds were tested in the NCI's human tumor, disease-oriented in vitro screen,[15] and data calculations were performed, as described elsewhere.[15-18] Figure 2 is a composite prepared directly from graphical information typically provided in the standard NCI screening data report package[18] and is representative of quadruplicate tests of **1** and **2**.

Screening Data Summary. The -log GI_{50}, TGI, and LC_{50} values, respectively, obtained directly from the original mean graphs provided in the standard NCI screening data report,[18] are listed below in sequence following the individual cell line names in the same order as the cell lines are arranged top-to-bottom on the mean graphs provided in the NCI supplier report.

Compound 1: CCRF-CEM (4.54, 4.16, 3.82), HL-60 TB (6.12, 5.36, 4.68), K-562 (5.92, 4.66, 3.85), MOLT-4 (4.68, 4.23, 3.85), RPMI-8226 (6.68, 6.15, 5.21), SR (5.47, 4.14, 3.89); A549/ATCC (5.54, 5.17, 4.72), EKVX (5.52, 5.24, 4.96), HOP-18 (5.49, 5.21, 4.92), HOP-62 (5.38, 5.05, 4.77), HOP-92 (6.14, 5.82, 5.44), NCI-H226 (4.62, 4.41, 4.21), NCI-H23 (4.85, 4.38, 4.00), NCI-H322M (5.68, 5.34, 5.02), NCI-H460 (5.82, 5.44, 5.08), NCI-H522 (4.62, 4.34, 4.06), LXFL-529L (4.85, 4.47, 4.12); DMS 114 (4.38, 4.12, 3.89), DMS 273 (5.96, 5.49, 5.10); COLO 205 (4.92, 4.70, 4.24), DLD-1 (5.74, 5.00, 4.46), HCC-2998 (5.77, 5.35, 5.00), HCT-116 (6.04, 5.49, 4.92), HCT-15 (5.66, 5.15, 4.62), HT29 (6.44, 5.85, 5.28), KM12 (6.17, 5.59, 5.16), KM20L2 (5.64, 5.33, 5.00), SW-620 (5.96, 5.52, 5.09); SF-268 (6.16, 5.54, 4.96), SF-295 (6.17, 5.70, 5.30), SF-539 (6.57, 6.19, 5.77), SNB-19 (5.70, 5.37, 5.04), SNB-75 (6.59, 5.80, 4.10), SNB-78 (6.60, 6.19, 5.70), U251 (6.40, 6.00, 5.64), XF498 (4.59, 4.30, 4.02); LOX IMVI (4.57, 4.24, 4.00), MALME-3M (4.57, 4.20, 3.89), M14 (4.57, 4.21, 3.92), M19-MEL (4.62, 4.24, 3.92), SK-MEL-2 (6.01, 5.49, 4.89), SK-MEL-28 (5.64, 5.32, 5.01), SK-MEL-5 (4.66, 4.38, 4.09), UACC-257 (5.68, 5.31, 4.89), UACC-62 (5.66, 5.17, 4.70); IGROV1 (4.82, 4.37, 3.96), OVCAR-3 (5.59, 5.33, 5.07), OVCAR-4 (5.57, 5.24, 4.85), OVCAR-5 (5.49, 5.24, 5.00), OVCAR-8 (5.72, 5.21, 4.66), SK-OV-3 (4.48, 4.21, 3.96); 786-0 (7.02, 6.66, 6.00), A498 (5.62, 5.34, 5.07), ACHN (5.85, 5.49, 5.15), CAKI-1 (5.80, 5.47, 5.05), RXF-393 (6.48, 6.06, 5.51), RXF-631 (6.47, 5.11, 4.52), SN12C (5.74, 4.77, 4.24), TK-10 (5.68, 5.37, 5.08), UO-31 (5.57, 5.19, 4.82).

Compound 2: CCRF-CEM (5.23, 4.43, >4.00), HL-60TB (5.09, >4.00, >4.00), K-562 (4.48, >4.00, >4.00), MOLT-4 (4.74, 4.12, >4.00); A549/ATCC (4.55, >4.00, >4.00), EKVX (4.42, >4.00, >4.00), HOP-18 (4.85, 4.55, 4.27), HOP-92 (4.80, 4.51, 4.24), NCI-H226 (4.57, 4.23, >4.00), NCI-H23 (4.82, 4.52, 4.21), NCI-H322M (4.29, >4.00, >4.00), NCI-H460 (4.96, 4.59, 4.19), NCI-H522 (4.96, 4.64, 4.31), LXFL 529 (4.92, 4.59, 4.28); DMS 114 (4.80, 4.48, 4.15), DMS 273 (4.74, 4.43, 4.12); COLO 205 (4.77, 4.52, 4.25), DLD-1 (4.82, 4.54, 4.28), HCC-2998 (4.82, 4.54, 4.26), HCT-116 (4.77, 4.51, 4.25), HCT-15 (4.80, 4.54, 4.27), HT29 (4.74, 4.48, 4.20), KM12 (4.44, >4.00, >4.00), KM20L2 (4.68, 4.36, 4.05), SW-620 (4.77, 4.49, 4.24); SF-268 (4.80, 4.39, >4.00), SF-295 (4.66, 4.24, >4.00), SF-539 (4.77, 4.49, 4.21), SNB-19 (4.33, >4.00, >4.00), SNB-75 (4.72, >4.00, >4.00); LOX IMVI (4.85, 4.54, 4.21), MALME-3M (5.00, 5.00, 5.00), M14 (4.89, 4.59, 4.29), M19-MEL (4.80, 4.47, 4.13), SK-MEL-2 (4.70, 4.44, 4.19), SK-MEL-28 (4.80, 4.52, 4.26), SK-MEL-5 (4.82, 4.54, 4.27), UACC-257 (4.82, 4.51, 4.21), UACC-62 (4.82, 4.55, 4.27); IGROV1 (4.82, 4.47, 4.13), OVCAR-3 (4.74, 4.48, 4.20), OVCAR-4 (4.43, >4.00, >4.00), OVCAR-5 (4.68, 4.44, 4.20), SK-OV-3 (4.49, >4.00, >4.00); 786-0 (4.72, 4.37, >4.00), A498 (4.64, 4.31, >4.00), ACHN (4.51, 4.03, >4.00), CAKI-1 (4.52, >4.00, >4.00), RXF-393 (4.82, 4.55, 4.28), RXF-631 (5.05, 4.59, 4.15), TK-10 (4.48, 4.01, >4.00), UO-31 (4.80, 4.54, 4.26).

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