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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · SEPTEMBER 2004

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In-vitro Cytotoxic Activities of the Major Bromophenols of the Red Alga *Polysiphonia lanosa* and Some Novel Synthetic Isomers

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Received December 9, 2003

Bioassay-guided fractionation was applied to the cytotoxic chloroform fraction of the red alga *Polysiphonia lanosa*. The major compounds of the most active fraction were identified using GLC-MS analysis as lanosol (**1**), methyl, ethyl, and *n*-propyl ethers of lanosol (**1a**, **1b**, and **1c**, respectively), and aldehyde of lanosol (**2**), although **1b** appears to be an artifact arising during the fractionation procedure. These compounds and other known bromophenols were synthesized in addition to four novel isomers (**3**, **3a**−**c**). The cytotoxic activities of all the synthetic compounds were determined against DLD-1 cells using the MTT assay. Compounds with IC_{50} < 20 μmol were also tested against HCT-116 cells. Compound **3c** (2,5-dibromo-3,4-dihydroxybenzyl *n*-propyl ether) was the most active compound against both cell lines (IC_{50} = 1.72 and 0.80 μmol, respectively), and its effect on the cell cycle was studied using flow cytometry.

Marine organisms are important and promising resources in cancer research, and a number of compounds from these organisms have undergone clinical trials as antitumor agents.[1]

In previous studies,[2,3] the methanolic extracts of 33 species of British marine algae were screened for in-vitro cytotoxic activities using DLD-1 cells (human colon adenocarcinoma). The methanolic extract of *Polysiphonia lanosa* (L.) Tandy was the only one found to have an IC_{50} value of less than 50 μg mL^{-1}, and its chloroform fraction was found to be significantly more active than the parent methanolic extract. *P. lanosa* is a marine red alga belonging to the Rhodomelaceae family, which grows epiphytically on other algae, primarily on the brown alga *Ascophyllum nodosum* (L.) Le Jol.[4] In common with many species of the Rhodomelaceae, *P. lanosa* is rich in brominated phenolic compounds, and a number of bromophenols have been isolated.[5,6]

Herein, we report the identification, synthesis, and cytotoxic activities of the major bromophenols of the chloroform fraction of *P. lanosa*, as well as the cytotoxic activities of some newly synthesized isomers.

Results and Discussion

The chloroform fraction of *P. lanosa* was nearly 10-fold more cytotoxic than the parent methanolic extract (Table 1); consequently the chloroform fraction was further fractionated by column chromatography over silica gel. One compound (**1a**) was isolated from fractions 6 and 7. The major compounds of the most active column fraction (F_{10}) in decreasing order of abundance, lanosol (**1**), the ethyl ether (**1b**) of lanosol, the aldehyde (**2**) of lanosol, the methyl ether (**1a**) of lanosol, and the *n*-propyl ether (**1c**) of lanosol, were identified by comparing the GLC-MS results of their silyl ethers with previously published data.[5,7,8] Compound **1b** was not detected in the parent chloroform fraction, but was detected in F_{10}, following column chromatography on silica gel in which EtOAc was used. This suggests that **1b** is an artifact most likely resulting from etherification of **1** by traces of EtOH in the EtOAc. The analogous formation of the methyl ether (**1a**) of **1** as an artifact resulting from the extraction of *P. lanosa* with MeOH has been previously reported.[5] As the amount of material available was insufficient to enable isolation of pure compounds, chemical synthesis was carried out.

Table 1. Cytotoxic Activities of *P. lanosa* Extract and Its Different Fractions against DLD-1 Cells and Activities of Synthetic Compounds against DLD-1 and HCT-116 Cells Using the 96 h MTT Assay

	IC_{50} ± SD, *n* = 3, vs DLD-1 cells		IC_{50} ± SD, *n* = 3, vs HCT-116 cells
extract/compound	μg mL^{-1}	μmol	μmol
MeOH extract	39.7 ± 1.5		NT[a]
$CHCl_3$ fraction	4.58 ± 0.79		NT
n-hexane fraction	>50		NT
aqueous fraction	>50		NT
F_9	2.77 ± 0.48		NT
F_{10}	2.35 ± 0.32		NT
F_{11}	4.19 ± 0.57		NT
1		18.3 ± 0.94	20.4 ± 2.9
1a		14.6 ± 3.1	14.1± 2.5
1b		13.5 ± 2.3	2.51± 0.95
1c		12.4 ± 1.1	1.32 ± 0.3
2		30.9 ± 2.7	NT
3		15.0 ± 2.0	21.4 ± 1.2
3a		7.27 ± 1.5	3.48 ± 0.63
3b		7.15 ± 1.4	1.54 ± 0.57
3c		1.72 ± 0.29	0.80 ± 0.63
4		70.0 ± 3.3	NT
5		27.9 ± 2.4	NT
6		31.0 ± 2.0	NT
7		6.43 ± 0.57	8.75 ± 0.13
9		>200	NT
10		91.3 ± 16	NT
11		51.5 ± 6.7	NT
11a		35.3 ± 9.5	NT
11b		35.1 ± 4.7	NT
11c		26.3 ± 4.2	NT
5FU[b]		7.38 ± 0.92	4.93 ± 0.81

[a] NT: not tested. [b] Positive control.

Bromination of protocatechualdehyde (**4**) ($Br_2/CHCl_3$ under reflux) (Scheme 1) produced the 5-bromo analogue **5** and 2,5-dibromo derivative **6** instead of the 5,6-dibromo compound **2** (for structure see Scheme 2) and **6**, as had been previously reported;[9] compounds **5** and **6** were recrystallized from EtOAc and MeCN, respectively. The structure

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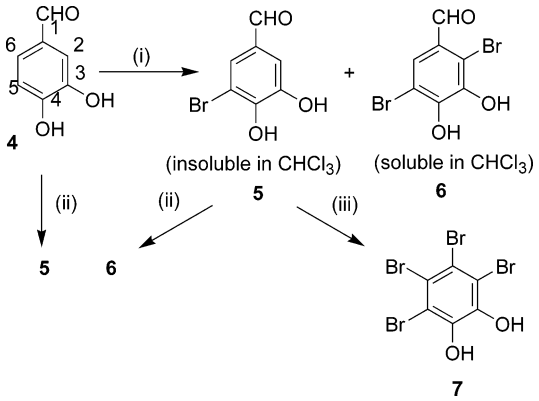
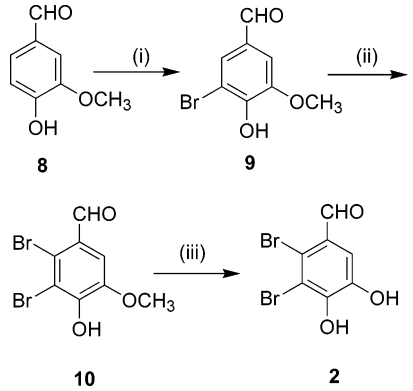
10.1021/np0305268 CCC: $27.50
Published on Web 07/09/2004

Scheme 1[a]



[a] (i) Br_2, $CHCl_3$, reflux, 15 h; (ii) Br_2, HOAc, rt, 30 min; (iii) Br_2, HOAc, reflux, 6 h.

Scheme 2[a]



[a] (i) Br_2, HOAc, rt, 45 min; (ii) Br_2, HOAc, Fe, reflux, 12 h; (iii) BBr_3.

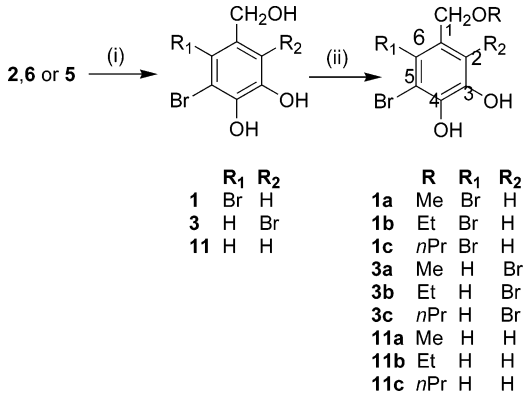
of monobrominated compound **5** was confirmed by MS, NMR, and GLC-MS data.[7,8]

Bromination of **4** using Br_2 and acetic acid[10] was initially unsuccessful. However, by modification of the workup, by pouring the reaction mixture into *n*-hexane instead of water, **5** was produced. Repeating the same procedure starting with **5** resulted in the partial conversion of **5** into **6**. Bromination of **5** using Br_2 and acetic acid under reflux (6 h), then pouring into *n*-hexane, produced needles of tetrabromocatechol (**7**); such oxidative replacement of a CHO group by Br_2 has been previously reported to take place using Br_2 in acetic acid[11] or with Br_2 in H_2O.[12] Bromination of vanillin (**8**) (Scheme 2) at room temperature gave 5-bromovanillin (**9**), which was recrystallized from EtOH. Further bromination of **9** by refluxing with Br_2 in the presence of Fe gave the 5,6-dibromovanillin (**10**). The spectroscopic data of **9** and **10** were consistent with the literature.[13,14] Several methods for the preparation of **2** by demethylation of **10** were tried. Trials using HBr/acetic acid[15] and chlorotrimethylsilane/sodium iodide[16] produced incomplete demethylation. However, complete demethylation was achieved with BBr_3.[17]

The alcohols **1**, **3**, and **11** were prepared from the corresponding aldehydes **2**, **6**, and **5**, respectively, by reduction with KBH_4[9] (Scheme 3).

Compound **1a** (32 mg) was prepared by heating a solution of **1** (40 mg) in 95% MeOH (10 mL) under reflux, until the reaction was completed (20 h), judged by monitoring with TLC. After evaporation of the solvent, the residue was recrystallized from CCl_4; **1b** and **1c** were prepared by reaction of **1** with EtOH and *n*-propanol, respectively. The remaining compounds (**3a**−**c**, **11a**−**c**) were prepared by the same procedures. The structures of the synthetic com-

Scheme 3[a]



[a] (i) KBH_4; (ii) MeOH, EtOH, or *n*-PrOH, reflux.

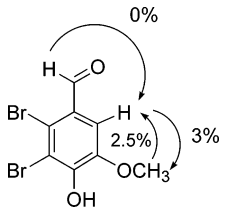


Figure 1. NOE enhancements confirming the regiochemistry of 5,6-dibromovanillin (**10**).

Table 2. GLC t_R of the Silylated Derivatives of Dibrominated Compounds

compound	t_R (min)	compound	t_R (min)
6	25.74	**2**	26.95
3	27.17	**1**	28.99
3a	25.17	**1a**	26.69
3b	26.06	**1b**	28.03
3c	28.07	**1c**	30.26

pounds were confirmed by MS, NMR, and GLC-MS, which were consistent with literature data.[7−9,13] NOE experiments showing dipolar coupling between the aromatic proton and the −OMe signal confirmed that the dibromovanillin (**10**) was the desired regioisomer (Figure 1). The MS of silylated derivatives of compounds **6**, **3**, and **3a**−**c** were nearly the same as those of compounds **2**, **1**, and **1a**−**c** present in F_{10}, respectively, but the t_R's are markedly different (Table 2). The MS of **6** and **2** have identical molecular ion peaks (*m*/*z* 298, 296, 294), but peaks at *m*/*z* 217, 215, due to loss of Br, were detected for **6** but not for **2**. Also ^{1}H NMR (DMSO-d_6) signals for the aromatic protons were at δ 7.29 and 7.55 for **2** and **6**, respectively, confirming that **6**, **3**, and **3a**−**c** are isomers of **2**, **1**, and **1a**−**c**, respectively.

The results of cytotoxicity tests (Table 1) against DLD-1 cells show that the activity is greatly influenced by the number and position of bromine substituents. The brominated derivative **5** is approximately 2-fold more active than the non-brominated compound **4**. The 2,5-dibrominated compounds showed higher activities than the corresponding 5,6-isomers (except for **2** and **6**, which showed similar activities). However, bromination was not the only factor affecting the activity, as brominated compounds with two phenolic groups (**2** and **5**) were found to be more active than those with one phenolic group (**10** and **9**), respectively. Also, the side chain influenced the activity; the most active compound of each series was the *n*-propyl ether derivative. The 2,5-dibrominated compound with an *n*-propyl side chain (**3c**) showed the highest activity against DLD-1 cells. Consequently this compound was further tested against DLD-1 cells using shorter exposure times (1, 3, and 24 h), and the IC_{50} values were 79.76, 77.98, and 2.71 μmol,

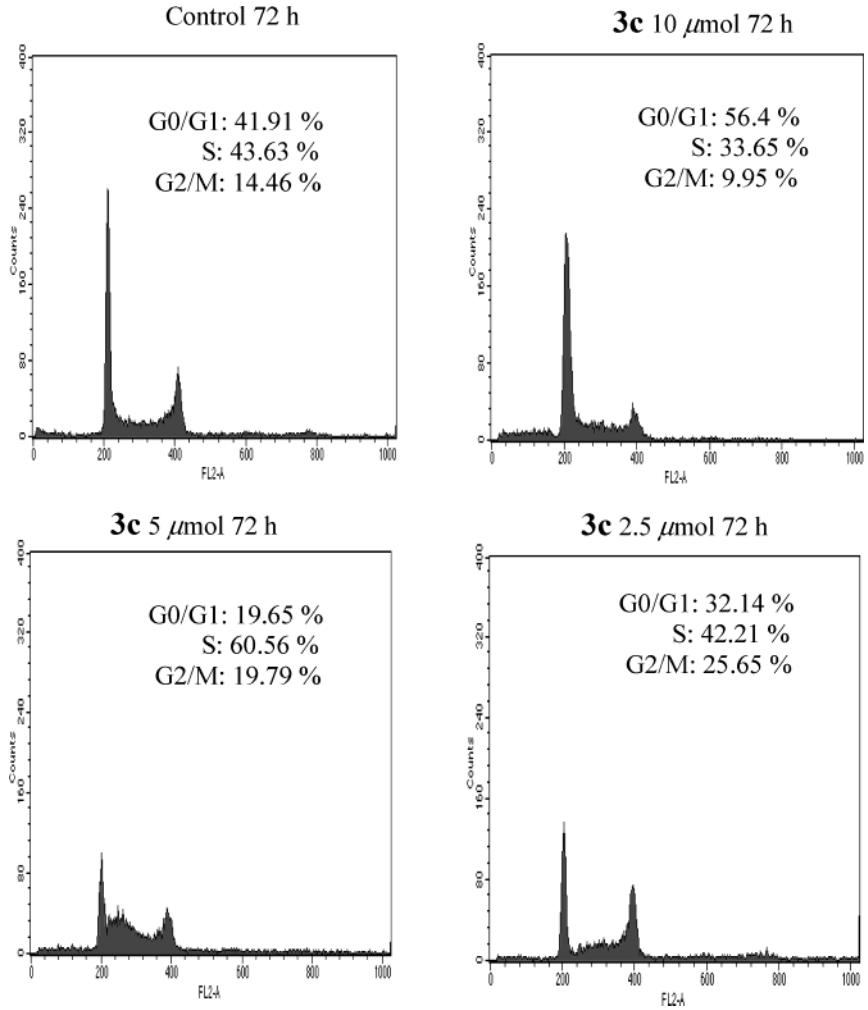


Figure 2. Effects of different concentrations of **3c** on cell cycle distribution of DLD-1 cells after 72 h drug exposure using flow cytometric analysis. In each histogram, the *y*-axis corresponds to the cell number and the *x*-axis refers to the FL-2 area. The cells in G0/G1 are represented by a peak at 200 on the *x*-axis, the cells in G2/M are represented by a peak at 400, and the area between these two peaks represents cells in S phase.

respectively, showing that cytotoxicity is greatly increased with longer exposure times. Although the IC_{50} values for **3c** at 24 and 96 h exposure were similar (2.71 and 1.72 μmol, respectively), it was observed that, with 96 h exposure, doses of 8 μmol and above killed nearly all the cells, whereas with 24 h exposure only a proportion of the cells was killed at the same doses. The compounds with IC_{50} values less than 20 μmol against DLD-1 cells were tested against another colon cell line, HCT-116, to confirm their activities. The IC_{50} value of compound **1c** against HCT-116 cells was nearly 10-fold higher than that against DLD-1 cells; however **3c** showed similar potency against both cell lines and was the most active compound in the series.

Some other brominated phenols (diphenyl ethers) isolated from the marine sponge *Dysidea herbacea* have been reported to have activity in the brine shrimp lethality test.[18] In common with the compounds reported here, the activity was influenced by the number of bromine substituents; similarly methylation of the phenolic hydroxyl groups resulted in weakening or loss of the activity in both groups of compounds.

Flow Cytometry. The most active compound, **3c**, was also investigated for its effect on the cell cycle. A range of concentrations (0.65−10 μmol) and exposure times (24, 48, 72, and 96 h) were used. The results of flow cytometry experiments are illustrated in Figure 2. At 10 μmol with all four exposure times, the proportion of cells in G0/G1 increased compared to the control cells ($P < 0.01$), showing that the cell cycle has been arrested at the G0/G1 phase. However, at 5 μmol the proportion of cells in S phase was significantly increased ($P < 0.001$), indicating S phase block. At 2.5 μmol a high proportion of cells in G2/M phase ($P < 0.05$) suggested inhibition at the G2/M phase. Concentrations lower than 2.5 μM had no significant effects on the cell cycle.

One possible explanation of the results could be that **3c** is affecting different phases of the cell cycle at different concentrations. It has previously been reported that some cyclin-dependent kinase (Cdk) inhibitors (pyridopyrimidine derivatives) at certain concentrations produced G1 arrest (inhibition of Cdk2 and Cdk4 kinases), while higher concentrations produced G2 arrest, possibly due to inhibition of Cdk1.[19] It is possible that **3c** is a nonspecific Cdk inhibitor, but further investigations will be needed to determine this. Another possible explanation is that the compound affects mainly the S phase (DNA synthesis), with high concentrations (10 μmol) acting at the G1/S boundary (early S), which appears as an increase in the number of cells at the G1 phase. With lower concentrations (5 μmol), the S phase is affected, and by decreasing the concentration further (2.5 μmol) the late S phase is inhibited, which appears as an increase in G2 phase cells.

Using an exposure time of only 1 h with high concentrations (100 and 50 μmol) a G0/G1 phase block resulted ($P < 0.001$, $P < 0.05$); concentrations less than 50 μmol showed

no significant effect. These results suggest inhibition of early DNA synthesis. The mechanism of action of **3c** cannot be completely understood from the above experiments, and other investigations are necessary such as multiparametric flow cytometry using bromodeoxyuridine,[20] Western analyses, and kinase assays.[21]

Experimental Section

General Experimental Procedures. GLC-MS analysis was carried out on a Hewlett-Packard 5890 series II gas chromatograph equipped with a 5972 A series mass selective detector and a 30 m capillary column, 0.25 mm internal diameter and 25 μm thickness, of stationary phase (Alltech-AT-5MS). Helium was used as the carrier gas at a linear velocity of 20 cm s^{-1}. The injector and detector temperatures were held constant at 280 and 310 °C, respectively. A solvent delay of 3.5 min and a temperature program from 100 to 300 °C at 4 °C min^{-1} were applied. EIMS were recorded on an Associated Electrical Industries Ltd. (AEI) MS 902 spectrometer. NMR spectra were acquired on a JEOL Delta GX270 (observing ^{1}H at 270.17 MHz and ^{13}C at 67.80 MHz) or JEOL ECA600 spectrometer (observing ^{1}H at 600.17 MHz and ^{13}C at 150.91 MHz). Melting points were carried out on an Electrothermal IA9000 series digital melting point apparatus. CHN analyses were determined using a Carlo Erba 1108 elemental analyzer at the University of Newcastle, U.K.

Alga Material. *P. lanosa* was collected from Kimmeridge Bay, Dorset, on the south coast of England in July 1999 and authenticated by one of us (G.B.). A voucher sample is deposited in the herbarium of the Hampshire County Council Museums Service, Winchester, Hampshire, U.K. (Index Herbariorum code HCMS; accession number Bi 2000.16.271). The alga (seaweed) was quickly dried at 50 °C and powdered.

Extraction and Fractionation. The dried powdered seaweed (5 g) was extracted three times with MeOH (100 mL) at room temperature, and the extracts were concentrated under reduced pressure, dried first under nitrogen and then under vacuum at room temperature. The dried extract was kept at 4 °C for cytotoxicity assay. The available amount (75 g) of *P. lanosa* powder of the same collection was successively extracted with *n*-hexane, followed by MeOH. The MeOH extract was then concentrated and partitioned between $CHCl_3$ and H_2O to afford 0.62 g of $CHCl_3$ residue.

The $CHCl_3$ fraction (0.5 g) was loaded onto a silica gel (45 g) column and eluted with *n*-hexane/EtOAc of increasing polarities to obtain nine fractions, each of 100 mL (F_1−F_9), followed by a gradient of EtOAc and MeOH to afford 11 fractions each of 100 mL (F_{10}−F_{20}). By performing TLC (silica gel GF_{254}, *n*-hexane/EtOAc/MeOH, 6.5:2.5:1) and spraying with 5% $FeCl_3$, no phenolic compounds could be detected in fractions F_1−F_5. Fractions F_6 (*n*-hexane/EtOAc, 3:2) and F_7 (*n*-hexane/EtOAc, 1:1) contained a single phenolic compound, which was further purified by recrystallization from CCl_4 to afford a yellowish white powder (3 mg). By spectroscopic analysis and GLC-MS, the compound was found to be the methyl ether (**1a**) of lanosol. [7] All other fractions were mixtures. Similar fractions were combined and tested for cytotoxic activity. Only three fractions {F_9, 6 mg (100% EtOAc), F_{10}, 25 mg, and F_{11}, 28 mg (EtOAc/MeOH, 9:1)} were found to have cytotoxic activity higher than that of the parent $CHCl_3$ fraction (Table 1).

GLC-MS. The most active fraction (F_{10}, IC_{50}: 2.35 μg mL^{-1}) was subjected to GLC-MS analysis. Identification of the bromophenols was made from the mass spectra of the trimethylsilyl derivatives after GLC.[8] The fraction (3 mg) was silylated by adding 40 μL of pyridine and 40 μL of BSTFA (*N*,*O*-bis-TMS-trifluoroacetamide containing 1% TMCS as catalyst) at 40 °C for 15 min.

Synthesis of Phenolic Compounds. Novel Synthetic Isomers. Note that signals for OH groups did not always appear in the ^{1}H NMR spectra.

2,5-Dibromo-3,4-dihydroxybenzyl Alcohol (3). KBH_4 (0.054 g, 1 mmol) dissolved in H_2O (1.2 mL) was added dropwise to a suspension of aldehyde **6** (0.296 g, 1 mmol) in 0.25 M phosphate buffer (pH 8, 3 mL). The mixture was stirred for 10 min and carefully acidified to pH 5 using dilute HCl. All reaction steps were performed in an ice bath to minimize side reactions. **3** (0.19 g, yield 64%) was extracted with $CHCl_3$ and precipitated upon concentration as a white powder: mp 132−134 °C; ^{1}H NMR (acetone-d_6) δ 4.58 (2H, s, ArCH_2), 7.25 (1H, s, 6-H), 8.45 (1H, br s, O*H*); ^{13}C NMR (acetone-d_6) δ 63.0 (ArCH_2), 108.5, 108.7, 121.8 (C-6), 134.0 (C-1), 142.2, 143.7; EIMS *m*/*z* 300 (23), 298 (48), 296 (25) $[M]^+$, 219 (38), 217 (45) $[M - Br]^+$, 190 (25), 188 (27) $[M - Br - HCO]^+$, 138 (12) $[M - 2Br]^+$, 110 (100) $[M - 2Br - CO]^+$, 53 (15); GLC-MS, t_R 27.17 min; EIMS of silylated derivative *m*/*z* 516 (5), 514 (9), 512 (4) $[M]^+$, 499 (3) $[M - CH_3]^+$, 435 (8), 433 (7) $[M - Br]^+$, 411 (7) $[M - OSi(CH_3)_3 - CH_2]^+$, 339 (24), 337 (47), 335 (24) $[M - OSi(CH_3)_3 - Si(CH_3)_3 - CH_3]^+$, 147 (11) $[(CH_3)_2Si{=}O{-}Si(CH_3)_3]^+$, 73 (100) $[Si(CH_3)_3]^+$; *anal*. C 28.52%, H 1.61%, calcd for $C_7H_6Br_2O_3$, C 28.20%, H 2.00%.

2,5-Dibromo-3,4-dihydroxybenzyl Methyl Ether (3a). A solution of **3** (50 mg, 0.17 mmol) in 95% MeOH (13 mL) was heated under reflux for 20 h when reaction was complete, as judged by monitoring with TLC (silica gel GF_{254}, *n*-hexane/EtOAc/MeOH, 6.5:2.5:1). After evaporation of the solvent, the residue (39 mg, yield 74%) was recrystallized from CCl_4 to afford yellowish white crystals: mp 106−107 °C; ^{1}H NMR (acetone-d_6) δ 3.35 (3H, s, CH_3), 4.39 (2H, s, ArCH_2), 7.14 (1H, s, 6-H); ^{13}C NMR (acetone-d_6) δ 57.6 (CH_3), 73.0 (ArCH_2), 108.5, 109.7, 123.0 (C-6), 130.6 (C-1), 142.8, 143.9; EIMS *m*/*z* 314 (29), 312 (63), 310 (31) $[M]^+$, 283 (49), 281 (100), 279 (51) $[M - OCH_3]^+$, 233 (80), 231 (81) $[M - Br]^+$, 53 (23), 31 (23); GLC-MS t_R 25.17 min; EIMS of silylated derivative *m*/*z* 458 (9), 456 (18), 454 (8) $[M]^+$, 425 (3) $[M - OCH_3]^+$, 339 (54), 337 (100), 335 (55) $[M - OCH_3 - Si(CH_3)_3 - CH_3]^+$, 289 (7) $[M - Br - Si(CH_3)_3 - CH_3]^+$, 73 (78) $[Si(CH_3)_3]^+$; *anal*. C 30.60%, H 2.20%, calcd for $C_8H_8Br_2O_3$, C 30.77%, H 2.56%.

2,5-Dibromo-3,4-dihydroxybenzyl Ethyl Ether (3b). A solution of **3** (50 mg, 0.17 mmol) in 95% EtOH (10 mL) was heated under reflux for 12 h (TLC monitored). Similar to **3a**, the recrystallized residue (46 mg, yield 83%) gave yellowish white crystals: mp 82−84 °C; ^{1}H NMR (acetone-d_6) δ 1.18 (3H, t, J = 7.05 Hz, CH_3), 3.55 (2H, q, J = 7.05 Hz, CH_2CH$_3$), 4.41 (2H, s, ArCH_2), 7.15 (1H, s, 6-H), 8.45 (1H, s, O*H*); ^{13}C NMR (acetone-d_6) δ 14.7 (*C*H$_3$), 65.7 (*C*H$_2$CH$_3$), 71.1(Ar*C*H$_2$), 108.4, 109.54, 122.8 (C-6), 131.0 (C-1), 142.7, 143.7; EIMS *m*/*z* 328 (19), 326 (40), 324 (20) $[M]^+$, 283 (44), 281 (91), 279 (46) $[M - OCH_2CH_3]^+$, 203 (89), 201 (100) $[M - OCH_2CH_3 - Br + H]^+$, 110 (66), 29 (57); GLC-MS t_R 26.06 min; EIMS of silylated derivative *m*/*z* 472 (8), 470 (15), 468 (7) $[M]^+$, 426 (4) $[M - CHOCH_3]^+$, 339 (47), 337 (88), 335 (45) $[M - OCH_2CH_3 - Si(CH_3)_3 - CH_3]^+$, 259, 257 (8) $[M^+ - OCH_2CH_3 - Br - Si(CH_3)_3 - CH_3 + H]$, 73 (100) $[Si(CH_3)_3]^+$; *anal*. C 32.58%, H 2.63% calcd for $C_9H_{10}Br_2O_3$ 0.08 CCl_4, C 32.24%, H 2.98%.

2,5-Dibromo-3,4-dihydroxybenzyl *n*-Propyl Ether (3c). Similar to above, a solution of **3** (70 mg, 0.23 mmol) in 95% *n*-propanol (11 mL) was heated under reflux for 6 h, and the recrystallized residue (67 mg, yield 86%), similarly, afforded pale brown crystals: mp 81−82 °C; ^{1}H NMR (acetone-d_6) δ 0.91 (3H, t, J = 7.39 Hz, CH_3), 1.58 (2H, sext, J = 7.39, 6.53 Hz, $CH_2CH_2CH_3$), 3.45 (2H, t, J = 6.53 Hz, OCH_2CH_2), 4.41 (2H, s, ArCH_2), 7.13 (1H, s, 6-H), 8.4 (1H, s, O*H*); ^{13}C NMR (acetone-d_6) δ 10.2 (*C*H$_3$), 22.8 (*C*H$_2$CH$_3$), 71.3 (O*C*H$_2$), 72.05 (Ar*C*H$_2$), 108.5, 109.6, 122.8 (C-6), 131.0 (C-1), 142.7, 143.8; EIMS *m*/*z* 342 (14), 340 (28), 338 (14) $[M]^+$, 283 (54), 281 (100), 279 (51) $[M - OCH_2CH_2CH_3]^+$, 219 (26), 217 (27) $[M - CH_2CH_2CH_3 - Br + H]^+$, 203 (40), 201 (50) $[M - OCH_2CH_2CH_3 - Br + H]^+$, 59 (20) $[OCH_2CH_2CH_3]^+$, 31 (73); GLC-MS t_R 28.07 min; EIMS of silylated derivative *m*/*z* 486 (7), 484 (12), 482 (6) $[M]^+$, 425 (6) $[M - OCH_2CH_2CH_3]^+$, 339 (45), 337 (87), 335 (44) $[M - OCH_2CH_2CH_3 - Si(CH_3)_3 - CH_3]^+$, 259, 257 (9) $[M - OCH_2CH_2CH_3 - Br - Si(CH_3)_3 - CH_3 + H]^+$, 73 (100) $[Si(CH_3)_3]^+$; *anal*. C 34.35%, H 3.19%, calcd for $C_{10}H_{12}Br_2O_3$ 0.1 CCl_4, C 34.13%, H 3.40%.

In-Vitro Cytotoxicity Assay. Human colon cell lines DLD-1[22] and HCT-116,[23] representatives of a common malignancy, were used, which are available at the Tom Connors Cancer Research Centre, Bradford University, U.K. These cell lines

are included in the NCI cell line panel and frequently used for screening potential anticancer agents. All cells were routinely maintained as monolayer cultures in RPMI 1640 medium with HEPES buffer (25 mmol) supplemented with fetal calf serum (10%), sodium pyruvate (1 mmol), L-glutamine (2 mmol), and penicillin/streptomycin (50 IU mL^{-1}/50 $\mu g\ mL^{-1}$). Chemosensitivity was assessed using the MTT assay.[24] Briefly, 1×10^4 cells mL^{-1} (180 μL) were inoculated into each well of a 96-well plate and incubated overnight at 37 °C in a humidified incubator (5% CO_2). All extracts/drugs were dissolved in DMSO and diluted in culture medium to provide a broad range of concentrations; the maximum DMSO concentration in any well was 0.1%. The medium was removed from each well and replaced with drug solutions (8 wells per drug concentration) and incubated at 37 °C for 4 days before the assessment of chemosensitivity. For short drug exposure times, the medium containing drug was removed after the required time and the cells were washed twice with medium; fresh medium was added (200 μL) and incubation continued to complete the 4 days. Culture medium was replaced with fresh medium (180 μL) prior to the addition of 20 μL of MTT solution (0.5 mg mL^{-1}). Following 4 h incubation at 37 °C, the medium plus MTT was removed from each well, and the formazan crystals were dissolved in DMSO (150 μL $well^{-1}$). The absorbance of the resulting solutions was determined at 550 nm using a Labsystems Multiskan Plus microplate reader. Results were expressed in terms of percentage survival, taking the mean absorbance of control samples to be 100% cell survival (Labsystems Genesis V3.04 software). Cytotoxicity was expressed as an IC_{50} value, which is the concentration of drug required to produce 50% inhibition of growth. 5-Fluorouracil (5FU), which is commonly used in the treatment of colon cancer, was used as a positive control. All experiments were performed in triplicate.

Analysis of Cell Cycle DNA by Flow Cytometry. Cell cycle analysis by flow cytometry was carried out using methodology adapted from that of Soni et al.[21] Briefly, DLD-1 cells (1.8 mL, 5×10^4 cells mL^{-1}) were seeded in 6-well plates. Following 24 h incubation at 37 °C, medium was removed from each well and replaced with drug solutions and incubated at 37 °C for 1, 24, 48, 72, and 96 h. For 1 h exposure, the cells were left to grow for 48 h before harvesting. The cells were harvested (trypsinisation and centrifugation), fixed with 70% EtOH, centrifuged, suspended in PBS, pH 7.3 (400 μL), treated with RNase (50 μL, 1 mg mL^{-1} in PBS), and stained with propidium iodide (50 μL, 400 $\mu g\ mL^{-1}$ in H_2O) for 30 min at 37 °C. Cellular DNA content was analyzed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA). Cells (10 000) were used for each analysis, and results were displayed as histograms. Percentages of cell population in each phase of the cell cycle were calculated with Cell Quest software (BDIS), WinMDi2.8 software, and Cylchred software.

Acknowledgment. N.A.S. is grateful to the Egyptian government for financial support. Mr. G. Dean (Geography and Environmental Science, University of Bradford, West Yorkshire, U.K.) is acknowledged for recording the GLC-MS data.

References and Notes

(1) Faulkner, D. J. *Antonie van Leeuwenhoek* **2000**, *77*, 135−145.
(2) Shoeib, N. A.; Bibby, M. C.; Cooper, P. A.; Blunden, G.; Wright, C. W. *J. Pharm. Pharmacol.* **2000**, *52 Suppl*, 311.
(3) Shoeib, N. A.; Bibby, M. C.; Cooper, P. A.; Blunden, G.; Wright, C. W. *BPC Sci. Proc.* **2001**, 170.
(4) Penot, M.; Hourmant, A.; Penot, M. *Physiol. Plant.* **1993**, *87*, 291−296.
(5) Stoffelen, H.; Glombitza, K. W.; Murawski, U.; Bielaczek, J.; Egge, H. *Planta Med.* **1972**, *22*, 396−401.
(6) Glombitza, K.-W.; Sukopp, I.; Wiedenfeld, H. *Planta Med.* **1985**, *5*, 437−440.
(7) Glombitza, K.-W.; Stoffelen, H.; Murawski, U.; Bielaczek, J.; Egge, H. *Planta Med.* **1974**, *25*, 105−114.
(8) Pedersen, M.; Saenger, P.; Fries, L. *Phytochemistry* **1974**, *13*, 2273−2279.
(9) Lundgren, L.; Olsson, K.; Theander, O. *Acta Chem. Scand.* **1979**, *B33*, 105−108.
(10) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*; Longman Scientific and Technical: New York, 1989; p 125l.
(11) Iyengar, M. S.; Jois, H. S. *J. Mysore Univ.* **1931**, *5*, 232−234.
(12) Anderson, E. *Am. Chem. J.* **1913**, 179−184.
(13) Kubo, I.; Ochi, M.; Shibata, K.; Hanke, F. J.; Nakatsu, T.; Tan, K.-S. *J. Nat. Prod.* **1990**, *53*, 50−56.
(14) Ford, P. W.; Narbut, M. R.; Belli, J.; Davidson, B. S. *J. Org. Chem.* **1994**, *59*, 5955−5960.
(15) Sudalai, A.; Rao, G. S. K. *Indian J. Chem.* **1989**, *28B*, 858−859.
(16) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247−1251.
(17) Yu, Q.-S.; Pei, X.-F.; Holloway, H. W.; Greig, N. H. *J. Med. Chem.* **1997**, *40*, 2895−2901.
(18) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R. W.; Kunzmann, A.; Soedarsono *J. Nat. Prod.* **1997**, *60*, 1313−1316.
(19) Soni, R.; Fretz, H.; Muller, L.; Schoepfer, J.; Chaudhuri, B. *Biochem. Biophys. Res.* **2000**, *272*, 794−800.
(20) Yamagami, K.; Matsubara, M.; Kitazawa, Y.; Takeyama, N.; Tanaka, T.; Kawamoto, K. *J. Appl. Toxicol.* **1994**, *14*, 155−159.
(21) Soni, R.; O'Reilly, T.; Furet, P.; Muller, L.; Stephan, C.; Zumstein-Mecker, S., Fretz, H.; Fabbro, D.; Chaudhuri, B. *J. Natl. Cancer Inst.* **2001**, *93*, 436−446.
(22) Dexter, D. L.; Barbarosa, J. A.; Calabresi, P. *Cancer Res.* **1979**, *39*, 1020−1025.
(23) Brattain, M. G.; Fine, W. D.; Khaled, F. M.; Thompson, J.; Brattain, D. E. *Cancer Res.* **1981**, *41*, 1751−1756.
(24) Mosmann, T. *J. Immunol. M.* **1983**, *65*, 55−63.

NP0305268