Cytotoxic Responses to Aromatic Ring and Configurational Variations in α-Conidendrin, Podophyllotoxin, and Sikkimotoxin Derivatives

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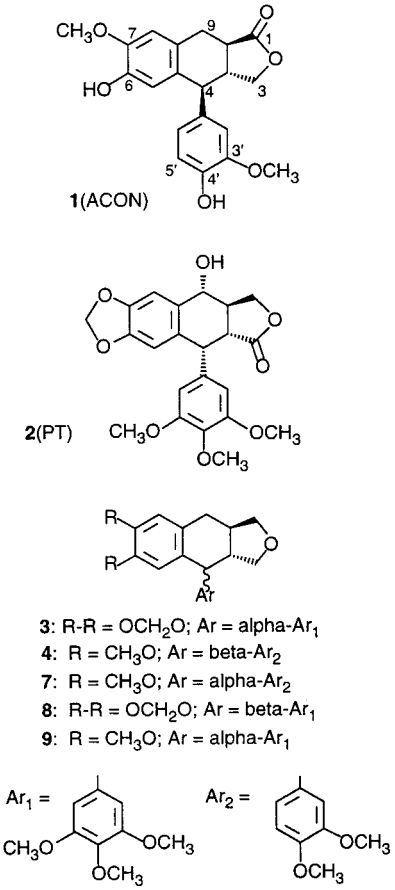
Derivatives of α-conidendrin, podophyllotoxin, and sikkimotoxin were prepared to evaluate the cytotoxic contributions of C-4 configuration and pendant and fused arene substitutions. Dimethyl-α-conidendryl alcohol (**5**), 9-deoxypodophyllol (**6**), and 9-deoxysikkimol (**17**) were dehydrated to their respective oxolane derivatives **4**, **3**, and **9**. Diols **5** and **6** were converted via oxabicyclo[3.2.1]octanols **10** and **14** to target oxolanes **8** and **7** where C-4 had been inverted relative to that in **3** and **4**. Cytotoxicities of the five oxolanes were determined in two drug-sensitive human leukemia and two multidrug-resistant cell lines expressing P-glycoprotein or multidrug-resistance associated protein (MRP). Changing the pendant arene configuration or replacing a *m*-methoxy by hydrogen resulted in a 100-fold cytotoxicity loss. Replacing a methylenedioxy group in the fused arene by two methoxy substituents reduced cytotoxicity by 10-fold. Drug-resistant cell lines were equally resistant to compounds **3**, **4**, **8**, and **9** indicating that these four compounds do not serve as substrates of the transport proteins P-glycoprotein and MRP.

Introduction

α-Conidendrin (ACON, **1**) (Chart 1) and podophyllotoxin (PT, **2**) are tetrahydronaphthalene (THN) lignans. ACON was once reclaimed on a large scale from sulfite pulping[1] and offered for commercial application. ACON seems to have no cytotoxic properties of current medical significance. In contrast, PT is a well-known cytotoxin isolated from *Podophyllum* species for manufacture of the oncolytic etoposide. Continued supply of PT from its source has been questioned.[2,3] In view of this background, we investigated the relative contributions of three structural differences between PT and ACON analogues that may account for dissimilar cytotoxicities of these lignans. In implementing this comparison, methylation of the two hydroxyl groups of ACON, hydrogenolysis of the C-9 hydroxyl group of PT, and conversion of the carbonyl groups of both ACON and PT to methylene groups were effected. The replacement of the hydroxyl group by hydrogen and the conversion of the carbonyl to a methylene group had transformed PT to the oxolane 9-deoxyanhydropodopodophyllol (**3**) with little bioactivity change.[4]

The remaining structural differences relate to the two arenes. The fused arene is substituted at C-6 and C-7 in both ACON and PT, by hydroxy and methoxy groups in ACON but by a methylenedioxy group in PT. The stereochemical configuration of the pendant arene at C-4 is β in ACON but α in PT. Also, ACON lacks the C-5′ methoxy group of PT. A group of PT derivatives bearing methoxy groups at C-6, C-7, and C-4′ showed no activity for DNA breakage or inhibiting topoisomerase II.[5] However, replacement of the C-5′ methoxy group of etoposide by hydrogen resulted in a cytotoxic derivative.[6]

Chart 1. Structures of γ-Lactone, Tetrahydronaphthalene Lignans, and Their Oxolane Analogues



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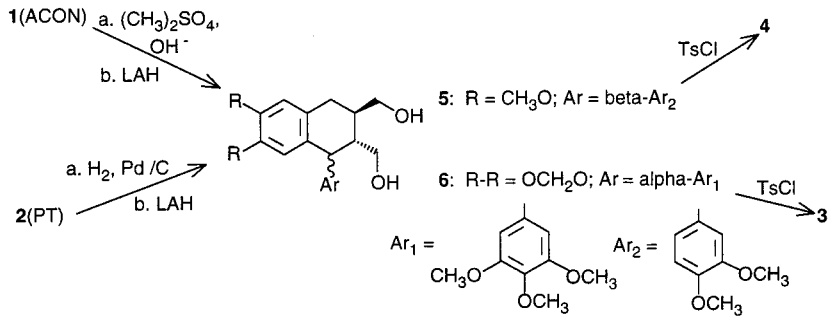
Scheme 1



Two of the five targets required for our study required inversion of C-4, from β to α in ACON and from α to β in PT. Anticipating these adjustments, we had investigated the influence of fused arene ring substituents that favored benzhydrylic (C-4) as opposed to benzylic (C-9) carbon oxygenation using simple THNs and β-conidendrol as models.[7] Here we report the preparation of the five PT and ACON targets required to separate the three structural variables and the results of the cytotoxicity assays required for the SAR analysis. In addition to our interest in cytotoxicity−SAR, the question of cell removal of the same PT and ACON analogues by P-glycoprotein (Pgp) and multidrug-resistance protein (MRP) pumps was addressed.

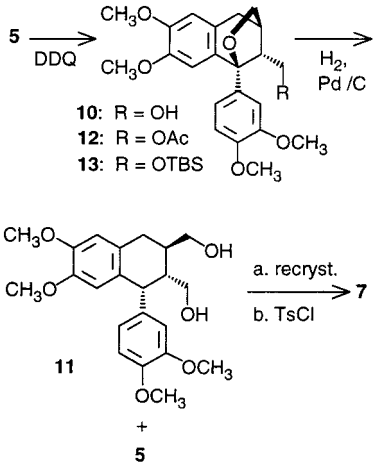
Chemical Transformations

ACON (**1**) and PT (**2**) were the source materials for five compounds required for comparing cytotoxic properties. The oxolane, dimethylanhydro-α-conidendrol (**4**) (Scheme 1), was obtained in three steps from **1**. Steps included methylation of the two phenolic hydroxyl groups of **1**, lithium aluminum hydride (LAH) reduction of the lactone to dimethyl-α-conidendrol (**5**) (Scheme 1), and tosyl chloride (TsCl)-promoted dehydration of the diol to **4**. Similarly, 9-deoxanhydropodophyllol (**3**) was obtained from **2** by Pd/C hydrogenolysis of the C-9 hydroxyl group of **2**, LAH reduction of the lactone to diol, **6**, and TsCl-promoted dehydration. Properties of oxolanes **3** and **4** were consistent with those previously reported[4,8] and the newly obtained spectral data.

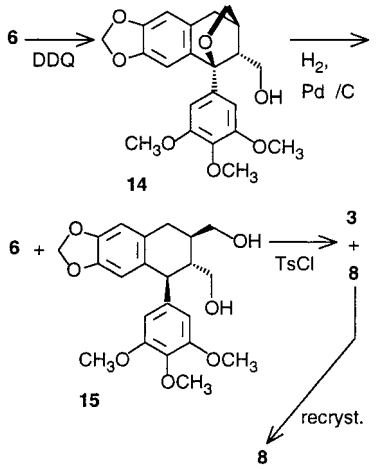
Inverting C-4 from its configuration found in the two source materials or replacement of the methylenedioxy group by two methoxy groups required additional intermediate steps to obtain the remaining three oxolanes **7**−**9** (Schemes 2−4). Inversion of C-4 in dimethyl-α-conidendryl alcohol (**5**) took advantage of the C-9a hydroxymethylene group's position and configuration. Treatment of **5** with 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ) in a mixture of CH_2Cl_2 and THF resulted in the intramolecular oxygenation of C-4 giving the oxabicyclo[3.2.1]octanol **10** (Scheme 2) as evidenced by DEPT, COSY, HETCOR, and HMBC analyses.

Since formation of oxabicyclooctanol **10** proceeded with inversion of configuration at C-4, succeeding hydrogenolysis of **10** required retention of configuration at C-4 for overall transformation of **5** to diastereomer **11** (Scheme 2), and ultimately to target **7**. However, the Pd/C-catalyzed hydrogenolysis of bicyclooctanol **10** produced diols **5** and **11** in a ratio of 58:42, as determined by HPLC and ^{1}H NMR. Likewise, Pd/C hydrogenolysis of the acetate ester **12** (Scheme 2) gave nearly equal

Scheme 2



Scheme 3



amounts of esters diastereomeric at C-4. However, hydrogenolysis of the *tert*-butyldimethylsilyl (TBS) ether **13** occurred with simultaneous loss of the silyl group and the required hydrogenolysis, giving nearly complete retention (93:7) favoring **11.** Recrystallization of the **11**-enriched mixture from acetone/hexane afforded pure **11**, which when treated with TsCl was converted to the oxolane **7**. Since hydrogenolyses of alcohol **10** and its ester **12** had occurred with undesired inversion, the bulky and less polar TBS derivative was chosen to shield the α-face of the molecule and favor catalyst contact from the less hindered β-face to give required retention of hydrogenolysis.

Preparation of the PT-derived oxolane **8** (Chart 1) involved DDQ-promoted conversion of diol **6** to oxabicyclooctanol **14** (Scheme 3). The structure assigned to

Scheme 4

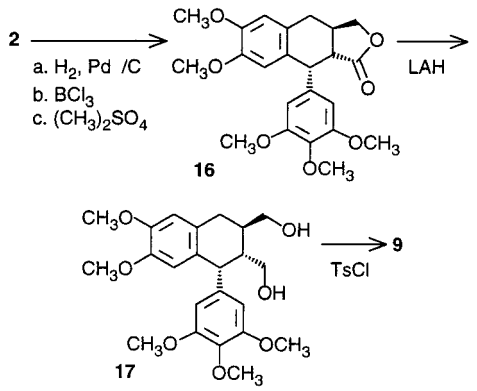


Table 1. Cytotoxicity of Tetrahydronaphthalene Lignan Derivatives to CCRF-CEM Cells

compd	IC_{50} (ng/mL)[a]	rel pot[b]
etoposide	1146 ± 83	1
3	43 ± 8	27.0
4	21862 ± 2511	0.05
7	38450 ± 3882	0.03
8	8147 ± 974	0.14
9	470 ± 38	2.4

[a] Data represent the mean ± SE of 2−4 independent experiments determined in duplicate. [b] Rel pot is the relative potency of the indicated compound compared to etoposide.

14 was consistent with ^1H and ^{13}C NMR, DEPT, COSY, HETCOR, and HMBC as well as assignments made for the similarly bridged structure **10**. The Pd/C hydrogenolysis of **14** produced a mixture of diastereomeric diols **6** and **15**. This mixture was transformed by TsCl dehydration to a mixture of oxolanes **3** and **8**, which on recrystallization from ether provided pure diastereomer **8**. The fifth oxolane required was **9** (Scheme 4). Its preparation began also with PT (**2**), which was converted in three steps to the 6,7-dimethoxy lactone analogue **16** of 9-deoxysikkimotoxin.[9] The lactone **16** was reduced to the diol **17**, which on TsCl dehydration provided oxolane **9**.

Cytotoxicity and SAR

Cytotoxicity was assessed using two human leukemic cells lines: CCRF-CEM (Table 1) and HL60 (Table 2). Table 2 also includes data for two multidrug-resistant variants: HL60/ADR and HL60/Vinc. These cells were selected for their resistance to vincristine or adriamycin and respectively overexpress either Pgp or MRP (Table 2). Both proteins are members of the superfamily of the ATP-binding cassette transport proteins. Overexpression of these proteins has been shown to confer resistance to a wide array of natural product drugs due to decreased accumulation of the cytotoxic agent intracellularly. The most cytotoxic of the six compounds to these cell lines was PT oxolane (**3**), which was approximately 30−50 times more active than etoposide. With respect to the remaining compounds, **3** was consistently 10−20 times more active than sikkimotoxin oxolane (**9**). Also, **3** was more active than the PT and ACON oxolanes **4**, **7**, and **8** by a factor of 10^2−10^3.

Each of the five oxolanes **3**, **4**, and **7**−**9** is distinguished by a unique set of three structural variables, which are: the configuration (α or β) of C-4, the number of methoxy groups (2 or 3) attached to the pendant arene, and the substitution of the fused arene by one methylenedioxy group or two methoxy groups. Oxolanes selected for pairwise comparisons of cytotoxicity were limited to those differing by a single variable. The mean cytotoxicities (Tables 1 and 2) for **8** and **3** show that a C-4 α- to β-configurational change results in an approximate activity loss of 100 in all four cell lines. Diminishing the number of pendant arene methoxy groups from three in **9** to two in **7** results in an activity loss of 100 as well. Replacement of the methylenedioxy group in the fused arene of **3** by the two methoxy groups in **9** produces a smaller 10-fold activity loss in all four cell lines. Although oxolanes **4** and **7** differ structurally by only the C-4 configuration, cytotoxicity for both has diminished substantially compared to **3** and to such a low level that cytotoxicities appear virtually the same.

As shown in Table 2, the HL60/ADR and HL60/Vinc cell lines are, respectively, 33- and 11-fold resistant to etoposide relative to HL60/S. In contrast, the resistant cell lines were more sensitive to the oxolanes by 2−5-fold. These data indicate that these oxolanes cannot be removed from the cell by either Pgp- or MRP-mediated efflux.

Conclusions

The configurations at C-4 in tetrahydronaphthalene lignans ACON and PT were inverted in three steps giving, respectively, two diols: **11** and **15**. These were the C-4 diastereomers of the corresponding two (**5** and **6**) obtained more directly by reduction of the lactones ACON and 9-deoxy-PT. Dehydration of the four diols resulted in four (**3**, **4**, **7**, and **8**) of the five lignan oxolanes required for the determination of cytotoxicities. The fifth resulted directly by replacement of the PT dioxymethylene group by two methoxy groups followed by routine reduction of lactone to diol and dehydration of the latter to the sikkimotoxin oxolane (**9**). Comparison of the cytotoxicities indicated that the greatest reduc-

Table 2. Cytotoxicities of Tetrahydronaphthalene Derivatives to Drug-Sensitive and -Resistant Cell Lines (HL60/S, HL60/ADR, and HL60/Vinc)[a,b]

	IC_{50} (ng/mL)					
compd	HL60/S	rel pot[c]	HL60/ADR	RF[d]	HL60/Vinc	RF[d]
etoposide	1643 ± 419		54940 ± 780	33	18088 ± 29	11
3	30 ± 1	54.8	19 ± 2	0.6	15 ± 0	0.5
4	36251 ± 8438	0.05	26054 ± 2763	0.7	14741 ± 725	0.4
7	117178 ± 771	0.01	23336 ± 2268	0.2	36083 ± 4120	0.3
8	7094 ± 75	0.23	4863 ± 225	0.7	3059 ± 467	0.4
9	533 ± 29	3.08	362 ± 5	0.7	314 ± 66	0.6

[a] HL60/ADR cells overexpress MRP and not Pgp; HL60/Vinc cells overexpress Pgp and not MRP. [b] Data represent averages of 2 determinations from a single experiment. [c] Rel pot is the relative potency of the compound compared to etoposide. [d] RF is the resistance factor of the cells to the compound where: RF = IC_{50}(drug-resistant cells)/IC_{50}(drug-sensitive cells).

tions in activity, relative to the PT oxolane **3**, resulted from two changes at the C-4 pendant arene. Inversion of C-4 from α to β and removal of one of two *m*-methoxy groups each reduced cytotoxicity by approximately 100-fold. In contrast, replacement of the dioxymethylene group by two methoxy groups in the fused arene diminished activity by 10-fold. We conclude that changes to the pendant arene attached to a rigid oxolane scaffold dramatically affect the potency of these compounds. In addition, these changes in structure also prevented their removal from the cell by two multidrug-resistant pumps, Pgp and MRP, and therefore may be potentially useful in the treatment of multidrug-resistant tumors. Since the five PT and ACON derivatives lack the glucoside and pendant ring phenolic hydroxy groups found in etoposide, it may be that the absence of these hydrophilic groups suppresses pumping of the five derivatives by Pgp and MRP.

Experimental Section

General. NMR data were obtained from Bruker 300 and 600 spectrometers and recorded in $CDCl_3$ solution, unless indicated otherwise. Chemical shift values (δ) are reported in ppm and in relation to TMS (δ 0.00) and $CDCl_3$ (δ 77.0) for ^1H and ^{13}C NMR, respectively; *J* values are in hertz (Hz). Quaternary, methine, methylene, and methyl carbons were differentiated by DEPT and when unassigned to a specific carbon are designated within parentheses as 0, 1, 2, and 3 in association with ^{13}C NMR δ values. $^1H-^1H$ correlation and one-bond $^1H-^{13}C$ connectivity were determined by COSY and HMQC or HETCOR experiments, respectively, while multiple-bond $^1H-^{13}C$ connectivity was established by HMBC. IR were obtained from deposited films on NaCl disks and are reported as absorbance in cm^{-1}. MS (low-resolution) were obtained by EI. HRMS were determined by the Midwest Center for Mass Spectrometry, University of Nebraska−Lincoln, NE. Preparative TLC was performed using 0.5- or 1.0-mm thickness silica gel plates containing fluorescent indicator and were viewed under 254-nm irradiation. Isocratic and gradient HPLC of **3**, **4**, and **7**−**9** submitted for cytotoxicity assays employed a 5-μm, C18(2), 250- × 4.60-mm column, with MeOH/H_2O (65/35, 1 mL/min) for isocratic analyses and CH_3CN/H_2O (50/50−95/5, 1 mL/min) in 25-min linear gradient analyses. Retention times (in min) are designated respectively t_{RI} for isocratic and t_{RG} for gradient analyses. Detection wavelength and temperature for all HPLC were 254 nm and 25 °C. Variation from these conditions is noted.

Materials. Growth medium and cell culture reagents were obtained from GIBCO (Grand Island, NY); FBS was purchased from Hyclone (Logan, UT). α-Conidendrin and podophyllotoxin were obtained from Crown Zellerbach (Camas, WA) and Bristol-Myers Squibb (Syracuse, NY) respectively, and etoposide was purchased from Sigma Chemical (St. Louis, MO).

General Reaction and Extraction Procedures. Unless indicated otherwise, reactions were conducted under dry N_2. Reaction solvents were dry and removed under vacuum after use. Reaction product extracts were dried over anhydrous $NaSO_4$ or $MgSO_4$ and the solvent was removed under vacuum.

General Procedure for Dehydrating the 1,4-Butanediol Lignans to the Corresponding Tetrahydrofurans. Following a known procedure[4] *p*-toluenesulfonyl chloride (TsCl) in pyridine was added to the 1,4-diol in pyridine, and the resulting mixture was heated to reflux for 3 h. Water was added, and the solution was extracted repeatedly with EtOAc. The combined EtOAc solution was washed successively with 1 M aqueous HCl, 5% aqueous $NaHCO_3$, water, and brine. From the organic layer resulted a residue that was purified by MPLC.

Preparation of 3 and 4 from Lignan Sources. α-Conidendrin was converted to dimethyl-α-conidendrin, which was reduced to dimethyl-α-conidendrol, **5**. Compound **5** was dehydrated to dimethylanhydro-α-conidendrol, **4**. **5**: mp 168−171 °C (lit.[8] 168−172 °C); $[\alpha]_D^{25}$ +33.2° (*c* 1.34, acetone) (lit.[8] $[\alpha]_D^{25}$ +21° (*c* 0.5, 95% EtOH)). **4**: mp 148−149 °C (lit.[8] 149−150 °C); $[\alpha]_D^{22}$ −36.8° (*c* 1.31, acetone) (lit.[8] $[\alpha]_D^{25}$ −52° (*c* 2.1, $CHCl_3$)); HPLC t_{RI} 12.1, t_{RG} 9.8. Anal. ($C_{22}H_{26}O_5$) H; C: calcd, 71.33; found, 70.73.

Podophyllotoxin was converted in three steps involving the successive intermediates 9-deoxypodophyllotoxin[4] and 9-deoxypodophyllol (**6**) leading to 9-deoxyanhydropodophyllol (**3**). **6**: mp 150−151 °C (lit.[4] 148−149 °C). **3**: mp 68−75 °C (amorphous solid) (lit.[9,10] 65−85 °C); $[\alpha]_D^{25}$ −59.5° (*c* 2.0, $CHCl_3$) (lit.[9,10] $[\alpha]_D^{25}$ −71° ($CHCl_3$)); HPLC t_{RI} 18.8, t_{RG} 12.0. Also, podophyllotoxin was converted in three steps through the known 9-deoxypodophyllotoxin[4] and 6,7-*O*-demethylene-9-deoxypodophyllotoxin[11] to the known 9-deoxysikkimotoxin (**16**),[12,13] which was reduced to 9-deoxysikkimol (**17**), and then dehydrated to obtain 9-deoxyanhydrosikkimol (**9**). Preparation procedures and properties of **17** and **9** are given further below. **16**: mp 151−153 °C (lit.[12] 162−163 °C (amorphous solid)); $[\alpha]_D^{25}$ −105.9° (*c* 1.7, $CHCl_3$) (lit. $[\alpha]_D$ −127° ($CHCl_3$),[12] $[\alpha]_D^{20}$ −85.8° (*c* 0.033, $CHCl_3$)[13]).

Conversion of Dimethyl-α-conidenrol (5) to Oxabicyclooctanol 10. To a stirred solution of 360 mg (0.93 mmol) of **5** in 54 mL of CH_2Cl_2/THF (39:15) at 25 °C was added 246 mg (1.08 mmol) of DDQ in 8.1 mL of CH_2Cl_2. Stirring continued 3 h and the solvents were removed. The residue was stirred with a mixture of 5% aqueous $NaHCO_3$ (33 mL) and EtOAc (30 mL), the phases were separated, and the EtOAc phase was washed with water then brine. The EtOAc phase was evaporated to dryness. Preparative TLC (CH_2Cl_2/EtOAc, 1:1) gave 171 mg (48%) of **10**: mp 65−72 °C; $[\alpha]_D^{25}$ +72.5° (*c* 1.71, acetone); IR 3500−3200; ^1H NMR ($CDCl_3$) (298 K) 6.87−7.4 (very broad singlet, 2, H-2′ and H-6′), 6.87 (d, J = 8.27, 1, H-5′), 6.66 (s, 1, H-5 or H-8), 6.45 (s, 1, H-8 or H-5), 4.24 (ddd, J = 8.10, 5.67, 2.24, 1, H-1), 3.89 (s, 3, OCH_3), 3.85 (s, 3, OCH_3), 3.84 (s, 3, OCH_3), 3.78−3.90 (m, 2, H-1, H-3), 3.69 (t, J = 10.76, 1, H-3), 3.59 (s, 3, OCH_3), 3.28 (m, 1, H-9), 2.89 (m, 1, H-9a), 2.78 (dd, J = 16.84, 2.11, 1, H-9), 2.29 (m, 1, H-3a); ^1H NMR ($CDCl_3$) (333 K) 7.13 (brs, 1, H-2′ or H-6′), 6.96 (brs, 1, H-6′ or H-2′) with the remaining signals being identical to those observed at 298 K; ^{13}C NMR ($CDCl_3$) 148.55 (0), 148.03 (0), 146.87 (0), 133.78 (C-1′), 131.40 (C-4a), 127.90 (C-8a), 119.12 (0), 112.00 (C-5 or C-8), 110.85 (C-8 or C-5), 110.45 (brs, C-5′), 83.78 (C-4), 72.55 (C-1), 59.91 (C-3), 55.92 (OCH_3), 55.87 (OCH_3), 55.76 (OCH_3), 53.34 (C-3a), 36.73 (C-9a), 32.75 (C-9). HRMS Calcd for $C_{22}H_{26}O_6$: 386.1729. Found: 386.1738.

Conversion of 9-Deoxypodophyllol (6) to Oxabicyclooctanol 14. 9-Deoxypodophyllol (123 mg, 0.31 mmol) in CH_2Cl_2/THF was treated with DDQ in CH_2Cl_2, using the procedure described for the preparation of **10**. Preparative TLC (CH_3Cl/EtOAc, 1:1) gave 51 mg (42%) of **14**: mp 148−149 °C; $[\alpha]_D^{23}$ +44.2° (*c* 1.19, acetone); IR, 3250−3550; ^1H NMR ($CDCl_3$) 7.16 (brs, 1), 6.64 (s, 1), 6.39 (s, 1), 6.18 (brs, 1), 5.88 (d, J = 1.39, 1, OCH_2O), 5.83 (d, J = 1.40, 1, OCH_2O), 4.23 (ddd, J = 8.14, 5.60, 2.46, 1, H-1), 3.97 (dd, 10.81, 5.48, 1, H-3), 3.70−3.89 (m, 11, H-1, H-3, 3(OCH_3)), 3.27 (td, J = 17.19, 2.76, 1, H-9), 2.85−2.90 (m, 1, H-9a), 2.75 (dd, J = 17.24, 2.23, 1, H-9), 2.32 (m, 1, H-3a), 1.72 (bs, 1); ^{13}C NMR ($CDCl_3$) 147.1 (0), 145.7 (0), 137.1 (0), 136.8 (0), 132.6 (0), 129.2 (0), 109.0 (C-5 or C-8), 107.7 (C-8 or C-5), 104.3 (brs, C-2′ and C-6′), 100.8 (OCH_2O), 84.1 (C-4), 72.6 (C-1), 60.8 (OCH_3), 60.0 (C-3), 56.2 (OCH_3), 52.8 (C-3a), 36.8 (C-9_a), 33.1 (C-9). HRMS Calcd for $C_{22}H_{24}O_7$: 400.1522. Found: 400.1526.

Conversion of Oxabicyclooctanol 10 to Acetate Ester 12. To a solution of **10** (100 mg, 0.26 mmol) in THF (100 mL) were added DMAP (65 mg, 0.53 mmol) and acetic anhydride (56 mg, 0.55 mmol) in THF (0.5 mL). The solution was stirred at 25 °C for 2 h. The THF was removed, and the residue was dissolved in ether, which was washed successively with 5% aqueous $NaHCO_3$ (2 × 6 mL), 1 M aqueous HCl (5 × 6 mL), water, and brine. The ether phase was dried, and the ether was removed giving 95 mg (85%) of **12**: mp 143.5−144.5 °C; $[\alpha]_D^{25}$ 101.5° (*c* 2.05, acetone); IR 1735; ^1H NMR (298 K) ($CDCl_3$) a very broad singlet (δ 6.9−7.4) appearing for 2

protons in the aromatic region; this signal sharpened somewhat when the NMR was recorded at 333 K; ^{1}H NMR (333 K) ($CDCl_3$) 7.14 (brs, 1), 6.98 (brs, 1), 6.87 (d, J = 8.37, 1), 6.67 (s, 1), 6.48 (s, 1), 4.32 (dd, J = 11.34, 5.45, 1), 4.23−4.28 (m, 1), 4.17 (dd, 11.24, 9.82, 1), 3.90 (s, 3), 3.87 (s, 3), 3.84 (s, 3), 3.83 (m, 1), 3.60 (s, 3), 3.21−3.28 (m, 1), 2.74−2.83 (m, 2), 2.41−2.48 (m, 1), 1.96 (s, 3); ^{13}C NMR ($CDCl_3$) 170.8 (0), 148.7 (0), 148.1 (0), 147.0 (0), 133.1 (0), 131.0 (0), 127.4 (0), 119.3 (0), 112.1 (1), 110.8 (1), 110.4 (1, brs), 83.8 (0), 72.5 (2), 61.9 (2), 55.91 (3), 55.87 (3), 55.76 (3), 49.6 (1), 36.9 (1), 32.8 (2), 20.7 (3). HRMS Calcd for $C_{24}H_{28}O_7$: 428.1835. Found: 428.1860.

Conversion of Oxabicyclooctanol 10 to *tert*-Butyldimethylsilyl Ether 13. *tert*-Butyldimethylsilyl chloride (413 mg, 2.74 mmol) in 3.6 mL of dry THF was added to a solution of **10** (288 mg, 0.75 mmol) and DMAP (378 mg, 3.09 mmol) in 6 mL of THF. The mixture was stirred at 25 °C for 24 h. Thereafter, 40 mL of 5% aqueous $NaHCO_3$ was added and the aqueous layer was extracted with EtOAc (5 × 20 mL). The combined EtOAc extracts were washed with water (2 × 70 mL) and brine (2 × 20 mL). Removal of EtOAc gave a residue that by MPLC (hexane/EtOAc, 4:1.5) yielded 349 mg (94%) of **13** as a viscous oil: $[\alpha]_D^{25}$ + 31.2° (*c* 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 6.88 (d, J = 8.40, 1), 6.68 (s, 1), 6.42(s, 1), 6.43−7.1 (brs of very low intensity, 2), 4.26 (ddd, J = 8.18, 5.60, 2.37, 1), 3.92 (s, 3), 3.87 (s, 1), 3.78−3.84 (m, 2), 3.63 (t, J = 10.31, 1), 3.60, (s, 3), 3.31 (m, 1), 2.89 (m, 1), 2.75 (dd, J = 17.20, 2.07, 1), 2.32 (m, 1), 0.84 (s, 9), − 0.02 (s, 3), − 0.04 (s, 3); ^{13}C NMR ($CDCl_3$) 148.4 (0), 147.88 (0), 146.77 (0), 133.91 (0), 131.77 (0), 128.26 (0), 119.26 (0), 111.95 (1), 110.67 (1), 110.42 (broadened, 1), 83.57 (0), 72.65 (2), 60.06 (2), 55.89 (3), 55.76 (3), 53.12 (1), 36.78 (1), 32.79 (2), 25.80 (3), 18.12(0), −5.39 (3), −5.52 (3). HRMS Calcd For $C_{28}H_{40}SiO_6$: 500.2594. Found: 500.25868.

Hydrogenolysis of Oxabicyclooctanol 10 to a Mixture of Dimethyl-α-conidendrol (5) and Dimethyl-4-iso-α-conidendrol (11). Catalyst 10% Pd/C: A solution of 60 mg of **10** in 10 mL of 95% EtOH was added to 30 mg of Pd/C, and the resulting suspension was stirred at 25 °C under H_2 for 6 h. The mixture was filtered and the EtOH was removed. TLC of the residue showed no **10**. HPLC revealed a ratio of **5** (t_R 19.97) to **11** (t_R 17.85) of 58:41, which was confirmed by ^{1}H NMR peak integrations. HPLC conditions were the same as those described in the general experimental conditions except the flow rate was 2 mL/min and the ratio of MeOH/H_2O = 1:1.

Catalyst Raney Ni: Similarly, 44 mg of catalyst, 16 mg (0.04 mmol) of **10** in 15 mL of ethanol stirred under H_2 for 72 h gave quantitative conversion to **5** and **11**, which appeared in a ratio of 78:21 by HPLC.

Hydrogenolysis of Acetate 12 to a Mixture of Dimethyl-α-conidendrol (5) and 4-Isodimethyl-α-conidendrol (11). Catalyst Pd/C: A solution of 63 mg (0.15 mmol) of **12** in 14 mL of 95% EtOH was stirred with 29 mg of Pd/C under H_2 for 24 h at 25 °C. Thereafter, TLC showed no starting ester. The suspension was filtered and EtOH was removed. The dry residue was chromatographed (CH_2Cl_2:EtOAc, 5:1) to obtain 55 mg (87%) of a mixture of two esters present in a ratio of 48:52 as indicated by HPLC (t_R 36.7 and 44.7). Methanolysis of the mixture in the presence of K_2CO_3 at 25 °C for 1.9 h produced, after workup, a mixture of **5** and **11** in a ratio of 46:54 by HPLC (t_R **5**, 20.0; **11**, 17.85). Conditions for HPLC were the same as those for the Pd/C hydrogenolysis of **10**.

Catalyst Raney Ni: Hydrogenolysis (1 atm) for 24 h of **12** (15 mg, 0.04 mmol) in dry EtOH (10 mL) in the presence of 11 mg of stirred catalyst resulted in complete conversion to a mixture of esters in a ratio of 44:56 by HPLC. Methanolysis of the mixture in the presence of K_2CO_3 gave **5** and **11** in a ratio of 44:56. Conditions for all the HPLC analyses were the same as those given for the Pd/C hydrogenolysis of **10**, above.

Hydrogenolysis of *tert*-Butyldimethylsilyl Ether 13 to Dimethyl-α-conidenrol (5) and Dimethyl-4-iso-α-conidendrol (11). *tert*-Butyldimethylsilyl ether **13** (318 mg, 0.64 mmol) in 91 mL of CH_3OH/hexanes (2.5:4) was added to 212 mg of 10% Pd/C. The suspension was stirred under H_2 for 6 h, and then filtered. Removal of the solvent gave 183 mg of crude product (74%). TLC indicated no **13**. HPLC (MeOH/H_2O, 1:1 at 1 mL/min) showed **11** (t_R 39.5) to **5** (t_R 44.7) ratio was 93:7. Recrystallization from acetone/hexanes gave 151 mg (61%) of **11**: mp 183−184 °C; $[\alpha]_D^{25}$ −276.7° (*c* 0.3, $CHCl_3$); IR 3500−3200; ^{1}H NMR 6.74 (d, J = 8.09, 1, H-5′), 6.65 (s, 1, H-8), 6.59−6.55 (m, 2, H-6′, H-2′), 6.37 (s, 1, H-5), 4.15 (brs, 1, H-4), 3.86 (s, 3, OCH_3), 3.83 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 3.83−3.76 (m, 2, H-3 and H-1), 3.69 (s, 3, OCH_3), 3.67 (m, 1, H-1), 3.53 (dd, J = 13.59, 5.08, 1, H-3), 2.89 (dd, J = 17.21, 4.38, 1, H-9), 2.76 (dd, J = 17.02, 9.90, 1, H-9), 2.13 (m, 2, H-9a and H-3a); ^{13}C NMR 148.52 (0), 147.81 (0), 147.56 (0), 135.71 (C-1′), 130.91 (C-8a or C-4a), 128.03 (C-4a or C-8a), 122.00 (C-6′), 113.38 (C-2′), 112.38 (C-5), 110.98 (C-8), 110.94 (C-5′), 65.44 (C-1), 65.08 (C-3); 55.97 (OCH_3), 55.85 (OCH_3), 47.71 (C-4), 43.68 (C-3a), 34.88 (C-9a), 32.58 (C-9). HRMS Calcd for $C_{22}H_{28}O_6$: 388.1886. Found: 388.18953.

Hydrogenolysis of Oxabicyclooctanol 14 to a Mixture of 4-Isodeoxypodophyllol (15) and Deoxypodophyllol (6). To a mixture of the benzoxabicyclooctane **14** (680 mg, 1.70 mmol) and 340 mg of 10% Pd/C were added 122 mL of ethanol and 14 mL of acetic acid. The resulting mixture was stirred under H_2 at a constant 50 °C for 8.5 h, cooled to 25 °C, and filtered. The filtrate was concentrated in vacuo to near dryness. The residue was extracted with EtOAc and the extract was washed with water then brine and dried. Removal of EtOAc gave a residue that was chromatographed successively with solvent CH_2Cl_2/EtOAc in ratios of 4:1 and 1:1, and finally with EtOAc. Removal of the EtOAc gave a white solid (523 mg, 76%), a mixture of **6** and **15** proving to be inseparable by HPLC using ODS columns and solvents of water/CH_3OH or water/CH_3CN. Therefore, the mixture was used in the dehydration step without separation.

Reduction of 9-Deoxysikkimotoxin (16) to 9-Deoxysikkimol (17). 16 (77.0 mg, 0.19 mmol) in 4 mL of THF was added dropwise to LAH (43.7 mg, 11.5 mmol) stirred in 2 mL of THF at 0 °C. Stirring was continued 3 h. Thereafter, EtOAc was added dropwise and then saturated aqueous NH_4Cl (3 mL) and water (1 mL). The mixture was filtered through sintered glass. The filtrate was extracted with EtOAc. The organic layer was washed with water and then brine. The EtOAc extract was dried, and the solvent was removed giving 63 mg (81%) of **17**: mp 125−127 °C; $[\alpha]_D^{25}$ −172° (*c* 1.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 6.64 (s, 1, H-8), 6.38 (s, 1, H-5), 6.24 (s, 2, H-2′, 6′), 4.13 (d, J = 3.57, 1, H-4), 3.86 (s, 3, OCH_3), 3.80 (s, 3, OCH_3), 3.74 (s, 6, OCH_3), 3.71 (s, 3, OCH_3), 3.90−3.70 (m, 2, H-1, 3), 3.64 (dd, J = 3.60, 10.50, 1, H-1), 3.51 (dd, J = 10.56, 6.45, 1, H-3), 2.87 (dd, J = 16.94, 4.87, 1, H-9), 2.75 (dd, J = 16.94, 10.41, 1, H-9), 2.40−2.20 (br s, 1, OH), 2.20−2.05 (m, 2, H-3a, 9a), 1.90−1.50 (br s, 1, OH); ^{13}C NMR ($CDCl_3$) 152.7 (C-3′, 5′), 147.8 (C-6), 147.5 (C-7), 138.6 (C-1′), 136.8 (C-4′), 130.5 (C-8a), 128.0 (C-4a), 112.4 (C-5), 111.0 (C-8), 107.3 (C-2′, 6′), 65.3 (2, C-1), 64.9 (C-3), 60.8 (OCH_3), 56.1 (OCH_3), 55.9 (OCH_3), 55.8 (OCH_3), 48.2 (C-3a), 43.6 (C-4), 34.9 (C-9a), 32.4 (C-9). HRMS Calcd for $C_{23}H_{30}O_7$: 418.1992. Found: 418.1977.

Dimethylanhydro-4-iso-α-conidendrol (7). TsCl (59 mg, 0.31 mmol) in 1 mL of pyridine was added to 63 mg (0.16 mmol) of **11** in 1 mL of pyridine. Heating the mixture, processing in the general manner, and MPLC (CH_2Cl_2/EtOAc, 5:1), gave 43 mg (72% yield) of **7** (white amorphous solid): $[\alpha]_D^{25}$ −156° (*c* 0.5, acetone); HPLC t_{RI} 10.0, t_{RG} 9.0; IR 1608; ^{1}H NMR 2.26−2.34 (m, 2, H-9a, H-3a), 2.59 (dd, J = 15.69, 10.91, 1, H-9), 3.00−3.08 (m, 2, H-3, H-9), 3.43 (dd, J = 9.46, 7.79, 1, H-1), 3.72 (s, 3, OCH_3), 3.77 (s, 3, OCH_3), 3.83 (s, 3, OCH_3), 3.88 (s, 3, OCH_3), 4.03 (dd, J = 15.28, 7.72, 2, H-1, H-3), 4.30 (d, J = 5.24, 1, H-4), 6.39 (dd, J = 8.16, 1.99, 1, H-6′), 6.43 (d, J = 1.97, 1, H-2′) 6.46 (s, 1, H-5), 6.67 (s, 1, H-8), 6.73 (d, J = 8.14, 1, H-5′); ^{13}C NMR 32.32 (C-9), 35.06 (C-9a), 44.84 (C-4), 46.07 (C-3a), 55.85 (OCH_3), 55.87 (OCH_3), 55.97 (OCH_3), 70.01 (C-3), 72.71 (C-1), 110.85 (C-5′), 111.46 (C-8), 113.50 (C-5), 113.60 (C-2′), 122.28 (C-6′), 128.54 (C-4a or C-8a), 130.65 (C-4a or C-8a), 134.68 (C-1′), 147.72 (0), 147.80 (0), 148.47 (0). HRMS Calcd for $C_{22}H_{26}O_5$: 370.17802. Found: 370.17863.

Deoxyanhydro-4-isopodophyllol (8). TsCl (710 mg, 3.72 mmol) and 500 mg (1.24 mmol) of a mixture of **6** and **15** in 10 mL of pyridine were heated. Workup in the general manner gave 285 mg of crude **3** and **8** which by a single recrystallization from ether gave 94 mg of **8** (24%): mp 171−172 °C; $[\alpha]_D^{25}$ −66.8° (*c* 0.66, acetone); HPLC t_{RI} 20.7, t_{RG} 12.2; ^{1}H NMR 2.24 (m, 2, H-3a and 9a), 2.74 (dd, J = 15.92, 9.65, 1, H-9), 2.98 (dd, J = 15.92, 3.97, 1, H-9), 3.48−3.56 (m, 2H), 3.68 (d, J = 9.53, 1), 3.81 (s, 6H, OCH_3), 3.82 (m, 1), 3.85 (s, 3, OCH_3), 4.19 (t, J = 7.26, 1, H-1), 5.87 (d, J = 1.29, 1, OCH_2O), 5.88 (d, J = 1.30, 1, OCH_2O), 6.30 (s, 1, H-5 or H-8), 6.31 (s, 2, H-2′ and H-6′), 6.61 (s, 1, H-8 or H-5); ^{13}C NMR 32.82 (C-9), 42.27 (C-9a), 50.56 (C-3a), 50.76 (C-4), 56.16 (OCH_3), 60.84 (OCH_3), 72.25 (C-3), 73.10 (C-1), 100.87 (OCH_2O), 105.30 (C-2′ and C-6′), 108.66 (C-8), 109.27 (C-5), 129.37 (C-4a), 132.80 (C-8a), 136.80 (C-4′), 140.27 (C-1′), 146.10 (C-6), 146.13 (C-7), 153.36 (C-3′ and C-5′). HRMS Calcd for $C_{22}H_{24}O_6$: 384.1573. Found: 384.1574. Anal. ($C_{22}H_{24}O_6$) C, H.

Dehydration of 9-Deoxysikkimol (17) to 9-Deoxyanhydrosikkimol (9). TsCl (54.7 mg, 0.29 mmol) in 3 mL of pyridine was added to a solution of **17** (60 mg, 0.14 mmol) in 3 mL of pyridine. Heating the mixture, workup in the general manner, and MPLC (CH_2Cl_2/EtOAc, 5:1) gave 37.0 mg (64.4%) of **9**, a glasslike solid liquifying at 100−101 °C: $[\alpha]_D^{25}$ −73.6° (*c* 2.7, $CHCl_3$); HPLC t_{RI} 8.7, t_{RG} 8.8; ^{1}H NMR ($CDCl_3$) 6.67 (s, 1, H-8), 6.47 (s, 1, H-5), 6.09 (s, 2, H-2′, 6′), 4.29 (d, J = 5.22, 1, H-4), 4.07 (dd, J = 7.70, 3.84, 1, H-1), 4.03 (dd, J = 15.12, 7.49, 1, H-3), 3.89 (s, 3, OCH_3), 3.81 (s, 3, OCH_3), 3.74 (s, 3, OCH_3), 3.73 (s, 6, OCH_3), 3.44 (dd, J = 9.35, 7.70, 1, H-1), 3.08 (dd, J = 10.58, 7.49, 1, H-3), 3.04 (dd, J = 15.94, 5.09, 1, H-9), 2.59 (dd, J = 15.94, 10.85, 1, H-9), 2.32 (m, 1, H-3a), 2.29 (m, 1, H-9a); ^{13}C NMR ($CDCl_3$) 152.8 (C-3′, 5′), 147.9 (C-6), 147.8 (C-7), 137.7 (C-1′), 136.9 (C-4′), 130.3 (C-8a), 128.6 (C-4a), 113.6 (C-5), 111.5 (C-8), 107.6 (C-2′, 6′), 72.8 (C-1), 70.1 (C-3), 60.8 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3), 55.9 (OCH_3), 46.0 (C-3a), 45.5 (C-4), 35.3 (C-9a), 32.3 (C-9). HRMS Calcd for $C_{23}H_{28}O_6$: 400.1886. Found: 400.1876.

Cell Culture. The human lymphoblastic leukemia cell line CCRF-CEM was obtained from Dr. W. T. Beck (Cancer Center, University of Illinois at Chicago, Chicago, IL). Cells were maintained in minimum essential media for suspension cultures containing Earle's salts, 2 mM L-glutamine, and 10% FBS.[14] The HL60 cells, an acute human myeloblastic leukemia, were also used in this study. The drug-sensitive parental HL60, the drug-resistant HL60/ADR which overexpresses MRP1, and HL60/Vinc which overexpresses Pgp were obtained from Dr. Melvin Center (Division of Biology, Kansas State University, Manhattan, KS). These cell lines were grown in RPMI 1640 media, containing L-glutamine and 10% FBS. Media for the HL60/ADR cells also contained 50 ng/mL doxorubicin.

Cytotoxicity Assays. Cell viability was determined using a modified tetrazolium dye reduction method.[15] Cells were harvested during logarithmic growth phase, seeded in 96-well plates (Costar) at 6.7 × 10^4 cells/well, and cultured in the presence of oncolytics as previously described.[16]

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Supporting Information Available: HPLC for **3**, **4**, and **7**−**9**; elemental analyses for **4** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Herrick, F. W.; Hergert, H. L. *Recent Advances in Photochemistry*; Plenum Press: New York, 1977; pp 443−515.
(2) Canal, C.; Moraes, R. M.; Dayan, F. A.; Ferreira, D. Podophyllotoxin. *Phytochemistry* **2000**, *54*, 115−120.
(3) Moraes-Cerdeira, R. M.; Burant, C. L., Jr.; Bastos, J. K.; Nanayakkara, N. P. D.; McChesney, J. D. In vitro propagation of *P. peltatum*. *Planta Med.* **1998**, *64*, 42.
(4) Gensler, W. J.; Murthy, C. D.; Trammell, M. H. Nonenolizable podophyllotoxin derivatives. *J. Med. Chem.* **1977**, *20*, 63.
(5) Wang, Z.-Q.; Hu, H.; Chen, H.-X.; Cheng, Y.-C.; Lee, K. H. Antitumor agents. 124. New 4*β*-substituted aniline derivatives of 6,7-*O*,*O*-demethylene-4′-*O*-demethylpodophyllotoxin and related compounds as potent inhibitors of human DNA topoisomerase II. *J. Med. Chem.* **1992**, *35*, 871−877.
(6) Saulnier, M. G.; Vyas, D. M.; Langley, D. R.; Doyle, T. W.; Rose, W. C.; Crosswell, A. R.; Lang, B. H. E-ring desoxyanalogues of etoposide. *J. Med. Chem.* **1989**, *32*, 1420−1425.
(7) Ramdayal, F. D.; Kiemle, D. J.; LaLonde, R. T. Directed DDQ-promoted benzylic oxygenations of tetrahydronaphthalenes. *J. Org. Chem.* **1999**, *64*, 4607−4609.
(8) Hearon, W. M.; MacGregor, W. S. The naturally occurring lignans. *Chem. Rev.* **1955**, *55*, 957−1068.
(9) Hartwell, J. L.; Schrecker, A. W. The Naturally Occurring Lignans. In *Progress in the Chemistry of Organic Natural Products*; Zechmeister, L., Ed.; Springer-Verlag: Vienna, 1958; Vol. 15, pp 83−166.
(10) Schrecker, A. W.; Hartwell, J. L. Application of tosylate reductions and molecular rotations to the stereochemistry of lignans. *J. Am. Chem. Soc.* **1955**, *77*, 432−437.
(11) Terada, T.; Fijimoto, K.; Nomura, M.; Yamashita, J.; Kobunai, T.; Takeda, S.; Wierzba, K.; Yamada, Y.; Yamaguchi, H. Antitumor agents. I. DNA topoisomerase II inhibitory activity and the structural relationship of podophyllotoxin derivatives as antitumor agents. *Chem. Pharm. Bull.* **1992**, *40*, 2720−2727.
(12) von Schreier, E. Zur structure des sikkimotoxins II. Partialsynthese der 6,7-dimethoxy-analogen von podophyllotoxin. (Toward the structure of the sikkimotoxins II. Partial synthesis of the 6,7-dimethoxy-analogues of podophyllotoxin.) *Helv. Chim. Acta* **1964**, *47*, 1529−1555.
(13) Coltart, D. M.; Charlton, J. L. The asymmetric synthesis of aryltetralin lignans: (−)-isolariciresinol dimethyl ether and (−)-deoxysikkimotoxin. *Can. J. Chem.* **1996**, *74*, 88−94.
(14) Beck, W. T.; Mueller, J. J.; Tanzer, L. R. Altered surface membrane glycoprotiens in Vinca alkaloid-resistant human leukemic lymphoblast. *Cancer Res.* **1979**, *39*, 2070−2076.
(15) Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1989**, *89*, 271−277.
(16) Dantzig, A. H.; Shepard, R. L.; Cao, J.; Law, K. L.; Ehlhardt, W. J.; Baughman, T. M.; Bumol, T. F.; Starling, J. J. Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberan modulator, LY335979. *Cancer Res.* **1996**, *56*, 4171−4179.

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