

Pierreiones A–D, Solid Tumor Selective Pyranoisoflavones and Other Cytotoxic Constituents from *Antheroporum pierrei*

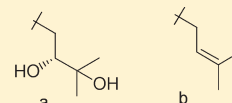
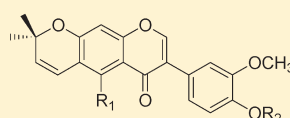
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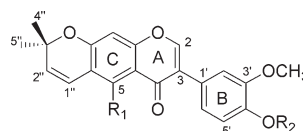
S Supporting Information

ABSTRACT: Bioassay-guided fractionation of a solid tumor selective extract of the leaves and twigs of *Antheroporum pierrei* acquired from the U.S. National Cancer Institute extract repository afforded four new pyranoisoflavones, pierreiones A–D (1–4), together with rotenone (5), 12a-hydroxyrotenone (6), and tephrosin (7). The structures of all new compounds were determined on the basis of their spectroscopic data, and the absolute configuration of 1 was assigned with the help of ¹H NMR analysis of its Mosher's ester derivatives. Compounds 1 and 5–7 accounted for the majority of the biological activity in terms of either cytotoxicity and/or selective toxicity to solid tumor cell lines. Pierreiones A (1) and B (2) demonstrated solid tumor selectivity with minimal cytotoxicity, while pierreione C (3) exhibited no activity.

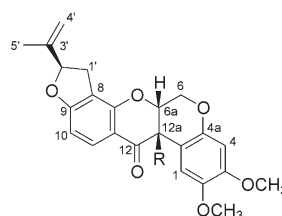
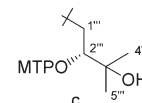
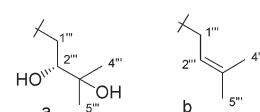


Pierreione A: 1 R₁=OCH₃, R₂=a
 Pierreione B: 2 R₁=H, R₂=a
 Pierreione C: 3 R₁=OCH₃, R₂=b
 Pierreione D: 4 R₁=H, R₂=b

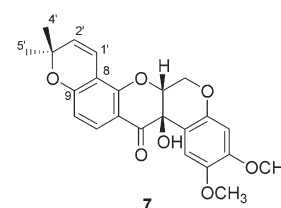
A major problem with present day cancer chemotherapy is the serious deficiency of drugs to treat solid tumors and the concurrent metastatic disease.¹ The majority of cell-based antitumor drug discovery efforts that have led to useful discovery of standard chemotherapeutic agents have relied frequently on their potency rather than selectivity. To address this deficiency, we have developed a high-throughput, cost-efficient, simple end-point disk diffusion soft agar assay based on differential clonogenic cytotoxicity between solid tumor cells and either normal or leukemia cells.² Solid tumor selectivity criteria in this approach incorporate both the cellular and molecular targets associated with the cancer phenotype. In continuing our efforts to uncover potential anticancer agents from plants,³ we investigated an extract of *Antheroporum pierrei* Gagnep. (Fabaceae) acquired from the natural products repository of the U.S. National Cancer Institute (NCI), which exhibited solid tumor selectivity in our assay. The genus *Antheroporum* Gagnep. contains five species, namely, *A. banaense*, *A. glaucum*, *A. harmandii*, *A. vidalli*, and *A. pierrei*.⁴ None of these species have been subjected to any detailed biological and/or chemical investigations, except for a report of the occurrence of the non-protein amino acids arginine and homoarginine in seeds of *A. pierrei*,⁵ a perennial tree native to China, Thailand, and Vietnam. Consequently, we investigated the solid tumor selective extract of *A. pierrei*, and herein we report the isolation and characterization of four new isoflavones, pierreiones A–D (1–4), and three known compounds, rotenone (5),⁶ 12a-hydroxyrotenone (6),^{6,7} and tephrosin (7),⁸ and in vitro evaluation of their potential anticancer activity.



1 R₁=OCH₃, R₂=a
 2 R₁=H, R₂=a
 3 R₁=OCH₃, R₂=b
 4 R₁=H, R₂=b
 8 R₁=OCH₃, R₂=c



5 R=H
 6 R=OH



Bioactivity-guided fractionation of the solid tumor selective CH₂Cl₂/MeOH (1:1) extract of leaves and twigs of *A. pierrei* afforded compounds 1–7. Pierreione A (1) was obtained as a colorless solid. Its molecular formula was established as C₂₇H₃₀O₈ from HRESIMS and ¹³C NMR data and indicated 13 degrees of unsaturation. The IR spectrum of 1 showed absorption bands for OH (3433 cm⁻¹) and conjugated CO

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(1639 cm^{-1}) functionalities. Its UV spectrum was typical of an isoflavone,^{9,10} and this was confirmed by the presence of characteristic NMR signals at δ_{H} 7.76 (s, H-2) and δ_{C} 150.6 (C-2).^{10–12} The ^1H NMR spectrum also showed four singlets due to methyl groups [δ 1.24 (3H), 1.29 (3H), and 1.46 (6H)], two OCH_3 groups [δ 3.86 and 3.87], and an aromatic proton (δ 6.59). In addition, it had signals due to an AB spin system consisting of two doublets ($J = 10.0$ Hz) at δ 6.72 and 5.71. This, together with the presence of a signal due to two methyl groups at δ 1.46 (6H, s), suggested that **1** contained a 2'',2''-dimethylpyran substituent.¹¹ The presence of this substituent was supported further by its ^{13}C NMR signals at δ_{C} 130.8, 116.0, 77.7, and 28.3.^{11,13,14} Long-range correlations observed in the HMBC spectrum of **1** (see Figure S17 in the Supporting Information) allowed placement of the aromatic proton (δ 6.59) at C-8 of ring A and one of the OCH_3 groups (δ 3.86) at C-5. In the NOE experiment, irradiation of the signal due to this OCH_3 caused enhancement of the olefinic proton at δ 6.72, suggesting that the dimethylpyran moiety is fused to ring A in a linear manner. In the aromatic region of the ^1H NMR spectrum, the presence of signals due to an ABX spin system [δ 7.14 (1H, d, $J = 2.0$ Hz), 6.97 (1H, dd, $J = 8.0$ and 2.0 Hz), and 6.93 (1H, d, $J = 8.0$ Hz)] suggested that ring B of this isoflavone was 1',3',4'-trisubstituted.¹⁰ The signal at δ_{H} 7.14 was assigned to H-2' due to its NOE with H-2 (see Figure S17 in the Supporting Information). The chemical shifts of H-6' (δ 6.97) and H-5' (δ 6.93) signals were in agreement with those reported for isoflavones.¹⁰ The NOE correlation observed between H-2' (δ 7.14) and the OCH_3 at δ 3.87 suggested that the latter group is attached to C-3'. The remaining signals in the ^1H NMR spectrum indicated the presence of an oxygenated methylene group [δ 4.29 (1H, dd, $J = 9.5$ and 2.5 Hz), 4.08 (1H, dd, $J = 9.5$ and 6.5 Hz)], an oxygenated methine group [δ 3.69 (1H, br s)], and two methyl groups [δ 1.29 (s) and 1.24 (s)] attached to an oxygenated carbon. These signals were assigned to a $\text{OCH}_2\text{CH}(\text{OH})\text{C}(\text{CH}_3)_2\text{O}$ spin system by comparison with the data reported for similar systems.^{15,16} Although no correlations were observed between C-1''' protons (δ 4.08 and 4.29) with the signal due to C-4' (δ 147.8) in the HMBC spectrum, this moiety was placed at C-4' based on its NOE data (see Figure S17 in the Supporting Information). The configuration at C-2''' was determined to be *R* by application of the modified Mosher's ester method according to the reported procedure (see Figure S18 in the Supporting Information).^{17,18} The structure of pierreione A was thus established as 3',5-dimethoxy-4'-(2*R*,3-dihydroxy-3-methylbutoxy)-3'',3''-dimethylpyrano-(6,7)-isoflavone (**1**).

The molecular formula of pierreione B (**2**) was determined as $\text{C}_{26}\text{H}_{28}\text{O}_7$ from its HRESIMS. The ^1H and ^{13}C NMR spectra of **2** closely resembled those of **1**, except that the OCH_3 group at C-5 was absent in **2**; instead it showed an additional aromatic proton (δ 7.85). The optical rotation of **2** was close to that of **1**, suggesting the *R* configuration at C-2'''. Thus, the structure of pierreione B was elucidated as 3'-methoxy-4'-(2*R*,3-dihydroxy-3-methylbutoxy)-3'',3''-dimethylpyrano-(6,7)-isoflavone (**2**).

Pierreione C (**3**) was obtained as a white, amorphous solid that analyzed for $\text{C}_{27}\text{H}_{28}\text{O}_6$ by HRESIMS. Its IR spectrum showed the presence of OH (3433 cm^{-1}) and conjugated CO (1640 cm^{-1}) groups. Compound **3** was also suspected to be an isoflavone based on its UV spectrum and the ^1H NMR signal due to H-2 at δ 7.76.^{9–12} In the ^1H NMR spectrum of **3**, the OCH_3 group at C-5 appeared at δ 3.87, while the signals of the 3'',3''-dimethylpyran

substituent on ring A were at δ 6.72 (1H, d, $J = 10.2$ Hz), 5.70 (1H, d, $J = 10.2$ Hz), and 1.45 (6H, s). Signals due to an ABX system at δ 7.11 (1H, d, $J = 1.8$ Hz), 6.90 (1H, d, $J = 7.8$ Hz), and 6.97 (1H, dd, $J = 7.8$ and 1.8 Hz) suggested that C-3' and C-4' of **3** were oxygenated.^{9,14} The ^1H NMR signal at δ 3.88 was assigned to an OCH_3 group (at C-3'). On the basis of literature precedence, the signals at δ 4.59 (2H, br d, $J = 6.6$ Hz), 5.51 (1H, t, $J = 7.0$ Hz), 1.75 (3H, s), and 1.72 (3H, s) were assigned to a 3'''-methyl-2'''-butenyl moiety linked to C-4' through an oxygen atom.^{19,20} The foregoing suggested that the structure of pierreione C should be identical to the dimethoxy derivative of the previously known pyranisoflavone, isoauricularin.²⁰ Thus, pierreione C was identified as 3',5-dimethoxy-4'-O-(3-methyl-2-butenyl)-3'',3''-dimethylpyrano-(6,7)-isoflavone (**3**).

Pierreione D (**4**), obtained as a white, amorphous solid, had the molecular formula $\text{C}_{26}\text{H}_{26}\text{O}_5$ based on its HRESIMS data. The ^1H NMR spectrum of **4** showed a very close resemblance to that of **3**, except for the presence of an aromatic proton signal at δ 7.86 in **4** instead of the signal due to the OCH_3 (δ 3.87) in **3**. The foregoing evidence identified pierreione D as 3'-methoxy-4'-O-(3-methyl-2-butenyl)-3'',3''-dimethylpyrano-(6,7)-isoflavone (**4**). Comparison of the spectroscopic and optical rotation data of compounds **5–7** with those reported in the literature allowed these to be identified as rotenone,⁶ 12a-hydroxyrotenone,^{6,7} and tephrosin,⁸ respectively.

A disk diffusion soft agar assay, using a panel of two solid tumor cell lines [C38 (colon adenocarcinoma) and HCT-116 (colon cancer)] and one leukemia cell line [L1210 (lymphocytic leukemia)], was used to evaluate the differential cytotoxicity (solid tumor selectivity) of the extract, fractions, and pure compounds.² IC₅₀ data for compounds **1–7** in the human colon cancer cell line HCT-116 were also determined. Taken together, these data (Table 2) suggested that pierreione A (**1**), rotenone (**5**), 12a-hydroxyrotenone (**6**), and tephrosin (**7**) accounted for the majority of the biological activity in terms of either cytotoxicity and/or selective toxicity to solid tumor cell lines, and pierreiones A (**1**) and B (**2**) demonstrated solid tumor selectivity with minimal cytotoxicity, while pierreione C (**3**) exhibited no activity. As the only difference between the structures of pierreione A (**1**) and pierreione C (**3**) is the presence of a 2,3-dihydroxyisopentane side-chain in ring C of **1** instead of an isopentenyl moiety in **3**, it is likely that the presence of the former is required for the cytotoxic activity of **1**. It is noteworthy that cytotoxic activities for rotenone (**5**),^{21–25} 12a-hydroxyrotenone (**6**),²³ and tephrosin (**7**)^{23,24} against several cancer cell lines have been reported previously.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Jasco Dip-370 polarimeter using CHCl_3 as solvent. IR spectra using KBr disks were recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in CDCl_3 with a Bruker DRX-500 and a Bruker DRX-600 instrument at 500 or 600 MHz for ^1H NMR and 125 MHz for ^{13}C NMR using residual CHCl_3 as the internal standard. Chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low-resolution and high-resolution MS were recorded on Shimadzu LCMS-DQ8000 α and JEOL HX110A spectrometers, respectively. HPLC was carried out on a Hitachi instrument consisting of a DAD and an ELSD detector. The semipreparative C-8 column (250 \times 10 mm, 5 μm) used was from Phenomenex Inc.

Table 1. ¹H NMR Data (500 or 600 MHz, δ, Hz) for Compounds 1–4 in CDCl₃

position	1 ^{a,b}	2 ^b	3 ^c	4 ^c
2	7.76 (s)	7.87 (s)	7.76 (s)	7.87 (s)
5		7.85 (s)		7.86 (s)
8	6.59 (s)	6.76 (s)	6.59 (s)	6.76 (s)
2'	7.14 (d, 2.0)	7.19 (d, 2.0)	7.11 (d, 1.8)	7.17 (d, 1.0)
5'	6.93 (d, 8.0)	6.93 (d, 8.0)	6.90 (d, 7.8)	6.90 (d, 8.0)
6'	6.97 (dd, 8.0, 2.0)	7.00 (dd, 8.0, 2.0)	6.97 (dd, 7.8, 1.8)	7.00 (br d, 8.0)
1''	6.72 (d, 10.0)	6.43 (d, 10.0)	6.72 (d, 10.2)	6.43 (d, 10.0)
2''	5.71 (d, 10.0)	5.73 (d, 10.0)	5.70 (d, 10.2)	5.72 (d, 10.0)
4''	1.46 (s)	1.47 (s)	1.45 (s)	1.47 (s)
5''	1.46 (s)	1.47 (s)	1.45 (s)	1.49 (s)
1'''	4.29 (dd, 9.5, 2.5)	4.30 (dd, 10.0, 2.0)	4.59 (br d, 6.6)	4.60 (br d, 6.6)
	4.08 (dd, 9.5, 6.5)	4.09 (dd, 10.0, 5.5)		
2'''	3.69 (br s)	3.70 (br s)	5.51 (t, 7.0)	5.51 (t, 7.0)
4'''	1.29 (s)	1.30 (s)	1.72(s)	1.72 (s)
5'''	1.24 (s)	1.29 (s)	1.75(s)	1.75(s)
5-OCH ₃	3.86 (s)		3.87(s)	
3'-OCH ₃	3.87 (s)	3.88 (s)	3.88(s)	3.89 (3H, s)

^aSignals were assigned by HMQC, HMBC, and ¹H–¹H COSY experiments. ^bAt 500 MHz. ^cAt 600 MHz.

Table 2. Zone Unit Differentials in the Disk Diffusion Soft Agar Colony Formation Assay^a and IC₅₀ Values for Compounds 1–7

compound	murine tumor selectivity		toxicity to human colon cancer cell line HCT-116
	conc (μg/disk)	Z _{C38} –Z _{L1210}	IC ₅₀ (μg/disk)
1	68	300	7.5
2	66	300	18
3	NA ^b		>46
4	1.9	600	30
5	0.23	500	0.045
6	0.26	450	0.055
7	0.24	550	0.040

^aMeasured in zone units: 200 zone units = 6 mm. Murine cell lines: C38 (colon adenocarcinoma), L1210 (lymphocytic leukemia). ^bNA = not active.

Plant Material. Leaves and twigs of *Antheroporum pierrei* were collected in Thailand in August 1987 for the U.S. National Cancer Institute (NCI) program on anticancer drug discovery and were identified by Prof. J. S. Burley. The NCI Open Repository Sample number N001507 was assigned to the sample.

Extraction and Isolation. Dried plant material was extracted with CH₂Cl₂–MeOH (1:1), residual solvents were removed under vacuum, and the extract (N001507) was stored at –20 °C in the NCI repository at the Frederick Cancer Research and Development Center (Frederick, MD). The crude extract (5.0 g sample from an NCI stock supply) was partitioned between hexanes and 80% aqueous MeOH. The bioactive MeOH(aq) fraction was diluted to 50% MeOH(aq) by the addition of water and extracted with CHCl₃. Evaporation of the CHCl₃ fraction yielded a dark green residue (474 mg), which was subjected to size-exclusion chromatography over Sephadex LH-20 (15.0 g) made up in hexanes/CH₂Cl₂ (1:4) and eluted with 250 mL each of hexanes/CH₂Cl₂ (1:4), CH₂Cl₂/acetone (3:2), CH₂Cl₂/acetone (1:4), and finally MeOH (500 mL). The bioactive hexanes/CH₂Cl₂ (1:4) fraction (248 mg) was subjected to column chromatography over Lichroprep diol (60 g) made up in hexanes/EtOAc (5:1) and eluted with hexanes/EtOAc (5:1, 500 mL), hexanes/EtOAc (3:1, 500 mL), hexanes/EtOAc (1:1, 500 mL), EtOAc (250 mL), EtOAc/MeOH (1:1, 250 mL), and MeOH (500 mL). The resulting fractions were combined on the basis of

their TLC profiles to afford 12 fractions (F1–F12). Of these, fraction F10 (78.8 mg), which was found to be active, was subjected to size-exclusion chromatography on Sephadex LH-20 (150 g) and eluted with CH₂Cl₂/MeOH (3:2) to afford three subfractions (F10a–c). The subfraction F10a (34.1 mg) was further fractionated on Sephadex LH-20 (150 g) and eluted with MeOH to obtain four subfractions, of which only the third fraction was found to be active. Compounds 1 (5.3 mg) and 2 (2.2 mg) were obtained from this active fraction by HPLC on a C-8 semipreparative column. Further fractionation of the active fraction F4 (4.3 mg) by HPLC under the same conditions afforded compounds 3 (2.3 mg) and 4 (0.9 mg). The active fraction F5 (29.2 mg) on Sephadex LH-20 (150 g) size-exclusion chromatography and elution with CH₂Cl₂/MeOH (3:2) yielded two subfractions, F5a and F5b. Further fractionation of the active subfraction F5b by HPLC as above afforded compounds 5 (1.4 mg), 6 (3.0 mg), and 7 (1.1 mg).

Pierreione A (**1**): white, amorphous solid; [α]_D²⁵ –5.4 (c 0.1, CHCl₃); UV (MeOH) λ_{max} (log ε) 325 (3.72), 271 (4.48), 228 (4.28), 203 (4.40) nm; IR (KBr) ν_{max} 3433, 2974, 2931, 1639, 1604, 1515, 1465, 1265, 1207, 1126, 1180, 1064 cm^{–1}; ¹H NMR data, see Table 1; ¹³C NMR (125 MHz, CDCl₃) δ 174.9 (C-4), 158.6 (C-9), 158.1 (C-7), 155.7 (C-5), 150.6 (C-2), 149.3 (C-3'), 147.8 (C-4'), 130.8 (C-2''), 126.1 (C-1'), 125.5 (C-3), 121.3 (C-6'), 116.0 (C-1''), 114.2 (C-5'), 113.3 (C-6), 113.1 (C-10), 113.0 (C-2'), 100.6 (C-8), 77.7

(C-3''), 74.9 (C-2'''), 71.9 (C-1'''), 71.9 (C-3'''), 62.8 (5-OCH₃), 55.9 (3'-OCH₃), 28.3 (C-4''), 28.3 (C-5''), 26.7 (C-4'''), 25.2 (C-5'''); HRESIMS *m/z* 483.2004 [M + H]⁺ (calcd for C₂₇H₃₁O₈, 483.2019).

Pierreione B (2): white, amorphous solid; [α]_D²⁵ −5.8 (c 0.1, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 331 (3.96), 267 (4.52), 228 (4.45), 206 (4.47) nm; IR (KBr) ν_{\max} 3425, 2974, 1639, 1621, 1515, 1477, 1269, 1203, 1141, 1110 cm^{−1}; ¹H NMR data, see Table 1; ¹³C NMR (125 MHz, CDCl₃) δ 175.6 (C-4), 158.0 (C-9), 157.4 (C-7), 152.1 (C-2), 149.4 (C-3'), 147.8 (C-4'), 131.7 (C-2''), 126.1 (C-1'), 124.5 (C-3), 123.7 (C-5), 121.2 (C-6'), 121.0 (C-1''), 114.2 (C-5'), 119.9 (C-6), 118.5 (C-10), 112.8 (C-2'), 103.9 (C-8), 77.9 (C-3''), 74.9 (C-2'''), 71.9 (C-1'''), 71.9 (C-3'''), 55.8 (3'-OCH₃), 28.5 (C-4''), 28.5 (C-5''), 26.7 (C-4'''), 25.2 (C-5'''); HRESIMS *m/z* 453.1872 [M + H]⁺ (calcd for C₂₆H₂₉O₇, 453.1913).

Pierreione C (3): white, amorphous powder; UV (MeOH) λ_{\max} (log ϵ) 325 (3.84), 272 (4.60), 228 (4.42), 206 (4.54) nm; IR (KBr) ν_{\max} 3433, 2927, 1639, 1640, 1512, 1461, 1265, 1207, 1126 cm^{−1}; ¹H NMR (600 MHz, CDCl₃) data, see Table 1; HRESIMS *m/z* 449.1968 [M + H]⁺ (calcd for C₂₇H₂₉O₆, 449.1964).

Pierreione D (4): white, amorphous powder; UV (MeOH) λ_{\max} (log ϵ) 331 (3.93), 267 (4.49), 228 (4.43), 204 (4.48) nm; IR (KBr) ν_{\max} 3461, 2931, 1647, 1612, 1517, 1473 cm^{−1}; ¹H NMR (600 MHz, CDCl₃) data, see Table 1; HRESIMS *m/z* 419.1853 [M + H]⁺ (calcd for C₂₆H₂₇O₅, 419.1859).

Rotenone (5): white solid; [α]_D²⁵ −173.3 (c 0.03, CHCl₃); LR-APCIMS and ¹H NMR data were consistent with literature values.⁶

12 α -Hydroxyrotenone (6): white solid; [α]_D²⁵ −90.7 (c 0.07, CHCl₃); LR-APCIMS, ¹H NMR, and ¹³C NMR data were consistent with those reported.^{6,7}

Tephrosin (7): white solid; [α]_D²⁵ −100.0 (c 0.09, CHCl₃); LR-APCIMS and ¹H NMR data were consistent with literature values.⁸

Preparation of the (R)- and (S)-MTPA Ester Derivatives (8a and 8b) of 1 by a Convenient Mosher Ester Procedure.^{18,19}

Compound 1 (1.0 mg) was transferred into a clean NMR tube and was dried under vacuum. Pyridine-*d*₅ (0.5 mL) and (S)-(-)- α -methoxy- α -(trifluoromethyl)phenylacetyl (MTPA) chloride (5 μ L) were added to the NMR tube immediately under a stream of N₂, and the tube was shaken carefully to mix the sample and the MTPA chloride. The NMR tube was allowed to stand at room temperature for 2 h to afford the (S)-MTPA ester derivative (8a) of 1. ¹H NMR data of 8a (600 MHz, pyridine-*d*₅): δ 8.08 (1H, s, H-2), 7.50 (1H, br s, H-2'), 7.27 (1H, d, *J* = 8.4 Hz, H-5'), 6.83 (1H, d, *J* = 10.2 Hz, H-1''), 6.03 (1H, d, *J* = 9.0 Hz, H-2'''), 5.76 (1H, d, *J* = 9.6 Hz, H-2''), 4.89 (1H, d, *J* = 11.4 Hz, H-1'''a), 4.56 (1H, dd, *J* = 11.4, 9.0 Hz, H-1'''b), 4.01 (3H, s, OCH₃, OCH₃-5 or OCH₃-3'), 3.80 (3H, s), 3.77 (3H, s, OCH₃-5 or OCH₃-3'), 1.43 (3H, s, H-4''' or H-5'''), 1.42 (6H, s, H-4'' and 5''), 1.37 (3H, s, H-4''' or H-5'''). In the manner described for 8a, another portion of 1 (0.9 mg) was reacted in a second NMR tube with (R)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (5 μ L) at room temperature for 2 h in pyridine-*d*₅ (0.5 mL) to afford the (R)-MTPA ester derivative (8b). ¹H NMR data of 8b (600 MHz, pyridine-*d*₅): δ 8.07 (1H, s, H-2), 7.50 (1H, d, *J* = 1.8 Hz, H-2'), 7.25 (1H, dd, *J* = 8.4, 1.8 Hz, H-6'), 7.12 (1H, d, *J* = 8.4 Hz, H-5'), 6.83 (1H, d, *J* = 10.2 Hz, H-1''), 6.77 (1H, s, H-8), 6.07 (1H, dd, *J* = 9.0, 1.8 Hz, H-2'''), 5.77 (1H, d, *J* = 10.2 Hz, H-2''), 4.76 (1H, dd, *J* = 10.8, 1.8 Hz, H-1'''a), 4.42 (1H, dd, *J* = 10.8, 9.0 Hz, H-1'''b), 4.00 (3H, s, OCH₃-5 or OCH₃-3'), 3.79 (3H, s), 3.77 (3H, s), 1.53 (3H, s, H-4''' or H-5'''), 1.52 (3H, s, H-4''' or H-5'''), 1.43 (6H, s, H-4'' and H-5'').

In Vitro Disk Diffusion Assay for Cytotoxicity. The disk diffusion assay was used to define differential cell killing among human and murine normal and malignant cell types as previously reported.² Since the initial extract (N001507) obtained from NCI was found to demonstrate selectivity between murine colon 38 and leukemia L1210 cells, these cell lines were used for bioassay-guided fractionation and

evaluation of the active compounds. Both the magnitude of the zonal difference and the potency was used for prioritization of fractions.

IC₅₀ Determinations. IC₅₀ studies were carried out against human HCT-116 colon cancer cells. These cells were grown in 5 mL of culture medium (RPMI-1640 + 15% FBS containing 1% penicillin–streptomycin and 1% glutamine) at 37 °C and 5% CO₂ at a starting concentration of 5 × 10⁴ cells/T25 flask. On day 3, cells were exposed to different concentrations of the drug. Flasks were incubated for 120 h (5 d) in a 5% CO₂ incubator at 37 °C, and the cells were harvested with trypsin, washed once with HBSS, resuspended in HBSS, and then counted using a hemocytometer. The results are normalized to an untreated control. The IC₅₀ values were determined using Prism 4.0 software.

■ ASSOCIATED CONTENT

S Supporting Information. ¹H NMR spectra for compounds 1–7, 8a, and 8b; ¹³C NMR spectra for compounds 1–3 and 6; ¹H–¹H COSY, HSQC, and HMBC spectra for compound 1; key HMBC and NOE correlations for 1 (Figure S17); $\Delta\delta$ values obtained for (S)- and (R)-MTPA esters (8a and 8b, respectively) of 1 (Figure S18). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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