Cytotoxic Styrylpyrones from *Goniothalamus amuyon*[1]

Yu-Hsuan Lan,[†] Fang-Rong Chang,[†] Jin-Hsien Yu,[†] Yu-Liang Yang,[†] Yi-Ling Chang,[‡] Shiow-Ju Lee,[‡] and Yang-Chang Wu*,[†]

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, and National Health Research Institute, Taipei, 114, Taiwan, Republic of China

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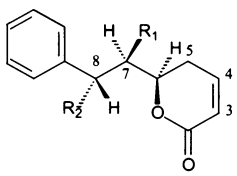
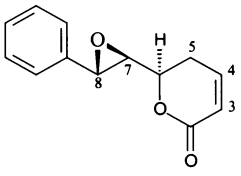
Two new styrylpyrones, (6*R*,7*R*,8*R*)-8-methoxygoniodiol (**1**) and (6*R*,7*R*,8*R*)-8-chlorogoniodiol (**2**), together with seven known styrylpyrones and eight other known compounds, were isolated from the leaves and/or stems of *Goniothalamus amuyon*. The structures of **1** and **2** were elucidated by spectral data interpretation, and the absolute stereochemistry of styrylpyrones in the diol and triol series was confirmed by X-ray crystallographic analysis and CD spectral data. Compound **2** demonstrated significant selective cytotoxicity toward the HONE-1 cancer cell line.

The genus *Goniothalamus* (Annonaceae) consists of 115 species, distributed throughout the tropics and subtropics,[2] with some used widely as traditional medicines.[3] Several bioactive styryllactones have been reported from *Goniothalamus* species.[4,5] The seeds of *G. amuyon* are reported to be useful for the treatment of edema and rheumatism.[6] In previous bioactivity-directed studies of the leaves of this species, the cytotoxic styrylpyrones goniodiol-7-monoacetate and goniodiol-8-monoacetate were isolated.[1] In the current reinvestigation of this medicinal plant, two new styrylpyrones, 8-methoxygoniodiol (**1**) and 8-chlorogoniodiol (**2**), together with eight known compounds, goniothalamin epoxide (**3**),[7] goniothalamin (**6**),[7,8] (5*S*,6*R*,7*R*,8*R*)-goniotriol (**7**),[1] (+)-9-deoxygoniopypyrone (**9**),[9] piperlactam C,[10] aristolactam FII,[11] and a mixture of β-sitosterol and stigmasterol,[12] were isolated from separate methanolic extracts of the stems and leaves of *G. amuyon*. (6*R*,7*R*,8*R*)-Goniodiol-7-monoacetate (**4**),[1] (6*R*,7*R*,8*R*)-goniodiol-8-monoacetate (**5**),[1] (5*S*,6*R*,7*S*,8*S*)-goniotriol (**8**),[12] liriodenine,[13] lysicamine,[14] (+)-pinoresinol,[15] veratric acid,[16] and cinnamic acid[17] were only obtained from the leaves. The structures of **1** and **2** were determined by interpretation of IR, ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra.



	R_1	R_2
1	OH	OCH_3
2	OH	Cl
4	OAc	OH
5	OH	OAc



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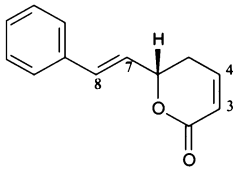

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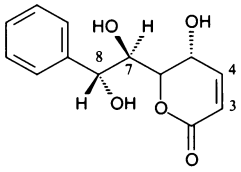

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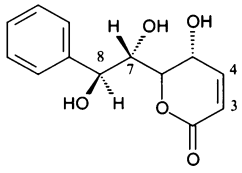

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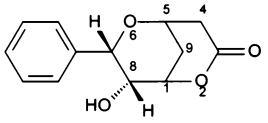

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Results and Discussion

Compound **1** was obtained as colorless crystals. In the EIMS, the molecular weight was indicated by a peak at *m*/*z* 248 $[M]^+$, and the presence of one hydroxyl and one methoxy group was suggested by peaks at *m*/*z* 230 $[M - H_2O]^+$, 217 $[M - OCH_3]^+$, and 198 $[M - H_2O - OCH_3]^+$. The molecular formula of **1** was established as $C_{14}H_{16}O_4$ by HRFABMS (found *m*/*z* 249.1121, calcd 249.1116). The IR absorption bands at 3419 and 1716 cm^{-1} suggested the presence of hydroxyl and carbonyl functions, respectively. In the ^{1}H NMR spectrum (Table 1), a singlet peak at δ 3.17 (3H, OCH_3) and a broad singlet peak at δ 2.22 (OH) were observed. The ^{1}H NMR signals at δ 7.28−7.38 (5H) represented a monosubstituted phenyl moiety, and the proton resonances at δ 5.98 (H-3) and 6.96 (H-4) and the carbon signals at δ 164.0 (C-2), 120.6 (C-3), and 145.9 (C-4) indicated an α,β-unsaturated δ-lactone moiety.[1] The C-2 resonance at δ 164.0 is a characteristic signal for an α,β-unsaturated δ-lactone in the styrylpyrones.[1] On the basis of the analysis of all of these data, the structure of **1** was assigned a styrylpyrone skeleton. Comparison of the NMR and MS data of **1** with those of goniodiol[1,9] indicated the structure of **1** to be 6-(β-methoxy-α-hydroxyphenethyl)-5,6-dihydro-2-pyrone. The 2D NMR spectra also supported the structural assignments proposed. The relative configuration at H-7 and H-8 could be determined by the $J_{7,8}$ coupling constant (8.8 Hz), which indicated an *erythro* form.[18] Compound **1** was subjected to single-crystal X-ray diffraction analysis (Figure 1). The optical rotation and X-ray crystal data suggested the relative configuration of **1**, and the absolute configuration was derived from its positive Cotton effect in the CD spectrum at 250−272 nm (n−π*).[19] Compared to goniothalamin,[20] the C-6 configuration was determined as being in the *R* form. The structure of **1** was established as 6*R*-(7*R*-hydroxy-8*R*-methoxy-8-phenyl)-5,6-dihydro-2-pyrone and was assigned the trivial name (6*R*,7*R*,8*R*)-8-methoxygoniodiol.

* To whom correspondence should be addressed. Tel: +886-7-312-1101, ext. 2197. Fax: +886-7-311-4773. E-mail: yachwu@kmu.edu.tw.
† Kaohsiung Medical University.
‡ National Health Research Institute.

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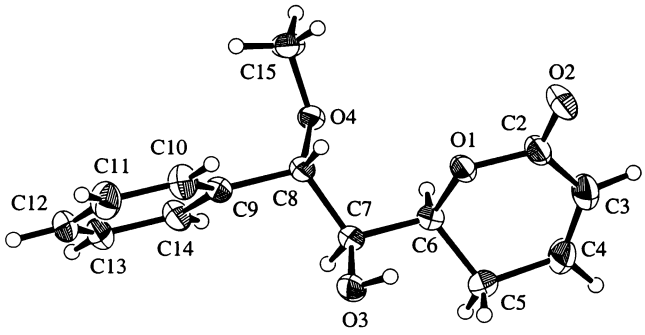
Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Spectral Data of Compounds **1** and **2** in $CDCl_3$[a]

	1		**2**	
position	δ ^{1}H (J in Hz)	δ ^{13}C	δ ^{1}H (J in Hz)	δ ^{13}C
2		164.0 (C)		163.5 (C)
3	5.98, dd (9.8, 2.4)	120.6 (CH)	6.05, dd (9.8, 2.6)	120.8 (CH)
4	6.96, ddd (9.8, 6.5, 2.4)	145.9 (CH)	6.98, ddd (9.8, 6.2, 2.0)	145.7 (CH)
5a	2.76, ddd (18.5, 13.0, 2.4)	25.9 (CH_2)	2.87, ddt (18.4, 12.8, 2.6)	26.2 (CH_2)
5b	2.25, ddd (18.5, 6.2, 4.0)		2.32, ddd (18.4, 6.4, 4.0)	
6	4.89, ddd (13.0, 4.0, 1.6)	76.1 (CH)	5.16, dd (12.8, 4.0)	75.8 (CH)
7	3.56, d (8.8)	81.7 (CH)	3.96, d (9.0)	75.4 (CH)
8	4.33, d (8.8)	74.6 (CH)	5.12, d (9.0)	60.0 (CH)
9	7.28−7.38, m	138.6 (C)	7.35−7.43, m	138.1 (C)
10		127.7 (CH)		128.1 (CH)
11		128.3 (CH)		128.9 (CH)
12		128.4 (CH)		129.0 (CH)
13		128.3 (CH)		128.9 (CH)
14		127.7 (CH)		128.1 (CH)
OCH_3-8	3.17, s	56.5 (CH_3)		

[a] All assignments were confirmed from 2D NMR spectra. Coupling constants in Hz are in parentheses, chemical shifts are in δ values.



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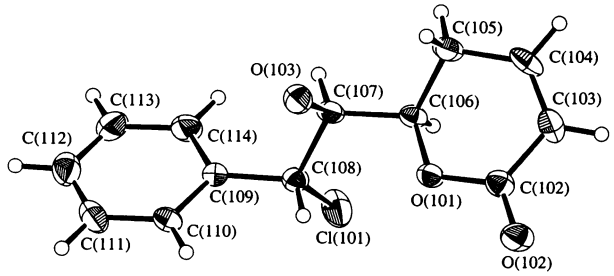

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Figure 1. ORTEP plots of (6*R*,7*R*,8*R*)-8-methoxygoniodiol (**1**) and (6*R*,7*R*,8*R*)-8-chlorogoniodiol (**2**)

Compound **2** was obtained as colorless plate crystals. The FABMS showed molecular ions at *m*/*z* 235 and 237 in the ratio 3:1 and indicated the presence of a chlorine atom in the molecule. The molecular formula, $C_{13}H_{13}ClO_3$, was confirmed by HRFABMS. IR bands at 3387 and 1714 cm^{-1} represented a hydroxyl group and a carbonyl group of an unsaturated δ-lactone, respectively. The ^{1}H NMR spectrum showed signals for five coupled aromatic protons of a monosubstituted phenyl moiety at δ 7.35−7.45, five methine protons at δ 3.90−7.00, and a pair of methylene protons at δ 2.28−2.90 (Table 1). These NMR signals were similar to those of **1**. The proton resonances of an α,β-unsaturated δ-lactone moiety at δ 6.05 (H-3) and 6.98 (H-4) and corresponding carbon signals at δ 163.5 (C-2), 120.8 (C-3), and 145.7 (C-4) were also present. Comparison of the ^{13}C NMR data of **2** with those of **1** revealed a few significant differences. Compound **2** lacked the ^{13}C NMR signal at δ 56.5 (OCH_3) observed in **1**, and the signal at δ 74.6 (C-8) in **1** was shifted upfield to δ 60.0 in **2**. Finally, the signal at δ 81.7 of **1** was shifted downfield to δ 75.4 in **2** (Table 1). The chemical shifts of C-6 in **1** and **2** were almost the same. These results suggested that the chloride atom was present at C-8 in **2**. Therefore, the planar structure of **2** was proposed as 6-(β-chloro-α-hydroxyphenethyl)-5,6-dihydro-2-pyrone. The 2D NMR spectra also confirmed these assignments. The relative configurations of H-7 and H-8 could be determined by the $J_{7,8}$ value (9.0 Hz), indicating an *erythro* form.[18] From the optical rotation value and X-ray crystallographic data of **2**, its relative configuration was confirmed. The absolute configuration of **2** was assigned as 6*R*,7*R*,8*R* by the optical rotation value, the X-ray crystallographic data, the circular dichroism spectrum [positive Cotton effect, 250−272 nm (n−π*)], and the similarity to biogenetically related derivatives.[1] Thus, the structure of **2** was determined as 6*R*-(7*R*-hydroxy-8*R*-chloro-8-phenyl)-5,6-dihydro-2-pyrone, which was named (6*R*,7*R*,8*R*)-8-chlorogoniodiol.

To establish that compounds **1** and **2** are not artifacts of the isolation procedure, the known compounds goniothalamin epoxide (**3**) and goniothalamin (**6**) were treated with 5% H_2SO_4 and $CHCl_3$ as a source of hydrochloric acid. One week later, from their NMR spectral data, these two products were not changed. According to these results, compounds **1** and **2** do not appear to represent the acid-catalyzed addition of water to goniothalamin epoxide (**3**) and the corresponding chlorohydrin, respectively.

The relative configuration of several styrylpyrones was confirmed by optical rotation value and X-ray crystallographic data. To check the absolute configuration, several diol- and triol-series styrylpyrones, namely, **1**−**5**, goniothalamin (**6**), (5*S*,6*R*,7*R*,8*R*)-goniotriol (**7**), (5*S*,6*R*,7*S*,8*S*)-goniotriol (**8**), and 9-deoxygoniopypyrone (**9**), were subjected to circular dichroism measurement (Supporting Information). The CD value of goniothalamin (**6**) showed a positive Cotton effect at 251 nm, due to the n−π* transition. In their CD spectra, all of the diol-series styrylpyrones appeared positive with absorptions at 251, 262, and 272 nm. The other chromophore, the benzene moiety, showed only minor changes in the CD spectra of diol styrylpyrones. Thus, C-6 can be assigned as the *R* configuration in this class. In the triol system, the CD data (positive at 250−270 nm) were compared with the literature data of (+)-osmundalactone, which has the same conjugated system of lactone moiety and relative configuration.[19] The CD data suggested that the stereochemistry of both triol styrylpyrones is 5*S* and 6*R*. Because the relative stereochemistry could be deter-

Table 2. Cytotoxic Activity of Styrylpyrones **1**−**5** against NUGC and HONE-1 Cell Lines

	IC_{50} (μg/mL)	
compound	NUGC[a]	HONE-1[b]
1	167.6 ± 12.5	239.7 ± 72.1
2	31.0 ± 9.70	4.87 ± 0.80
3	32.1 ± 4.91	36.3 ± 6.62
4	4.12 ± 0.71	5.69 ± 0.67
5	5.02 ± 0.43	6.09 ± 1.58
actinomycin D	6.61 ± 1.51	4.53 ± 1.14

[a] NUGC: Human gastric cancer. [b]HONE-1: Human nasopharyngeal carcinoma.

mined by NMR, the absolute configurations of the two known triols were assigned as (5*S*,6*R*,7*R*,8*R*)-goniotriol (**7**) and (5*S*,6*R*,7*S*,8*S*)-goniotriol (**8**), respectively. The benzene moiety also has no great effect in the CD spectra of the triol styrylpyrones.

The known compounds from *G. amuyon* were identified by comparison of their spectral and physical data with authentic compounds and values reported in the literature. It is interesting to note that the acetyl styrylpyrones, including goniodiol-7-monoacetate and goniodiol-8-monoacetate, were isolated only from the leaves of this species but were the major secondary metabolites.

Among the isolates, **1**, **2**, goniothalamin epoxide (**3**), goniodiol-7-monoacetate (**4**), and goniodiol-8-monoacetate (**5**) were subjected to evaluation in a cytotoxicity assay (Table 2). Compound **1**, with an 8-methoxyl substitution, showed a reduced activity relative to the other substances tested. Compound **2** is the first styrylpyrone possessing a chlorine atom and was cytotoxic for only the HONE-1 (human nasopharyngeal carcinoma) cell line in the present investigation. As predicted from previous results,[1] the known compounds goniodiol-7-monoacetate (**4**) and goniodiol-8-monoacetate (**5**) also showed significant cytotoxicity against the NUGC (human gastric cancer) and HONE-1 cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Mattson Genesis II spectrophotometer. CD spectra were recorded on a JASCO J-810 spectrometer. ^{1}H NMR (400 MHz, using $CDCl_3$ as solvent for measurement), ^{13}C NMR (100 MHz), HETCOR, DEPT, and NOESY spectra were obtained on a Varian NMR spectrometer (Unity Plus). Low-resolution EIMS were collected on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC/MS spectrometer having a direct inlet system. High-resolution FABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography, while TLC analysis was carried out on Si gel GF_{254} precoated plates with detection using 50% H_2SO_4 followed by heating on a hot plate.

Plant Material. Fresh leaves and stems of *G. amuyon* (Blanco) Merr. were collected in Hengchun, Pingtung Hsien, Taiwan, in September 1999 (Goniothalamus 1) and September 2001 (Goniothalamus 2). They were identified by Dr. Hsin-Fu Yen (National Museum of Natural Science, Taichung, Taiwan). The two voucher specimens are deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. Fresh stems (7.4 kg) of *G. amuyon* were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated under reduced pressure to give a syrup, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to give a residue (180 g), which was extracted with 5% H_2SO_4 to give a neutral $CHCl_3$ layer and an acidic aqueous solution. The latter was basified with NH_4OH and extracted with $CHCl_3$ to afford a basic alkaloid fraction (25 g). The neutral $CHCl_3$ solution was dried and evaporated to leave a brownish viscous residue (125 g). The neutral $CHCl_3$ residue was subjected to Si gel (2.5 kg, 53 × 11 cm) column chromatography and eluted with gradually more polar *n*-hexane/$CHCl_3$/EtOAc/MeOH mixtures; the eluents were combined into 26 fractions on the basis of TLC. Fraction 4 was eluted with $CHCl_3$ to give a mixture of β-sitosterol and stigmasterol[12] (30 mg). Fractions 6−8 were eluted with $CHCl_3$ to give goniothalamin (**6**)[7,8] (120 mg). Fraction 10 was eluted with *n*-hexane/EtOAc (5:1) to give aristolactam FII[11] (4 mg). Fractions 11 and 12 were purified by repeated Si gel column chromatography to afford **2** (50 mg), piperlactam C[10] (5 mg), and goniothalamin epoxide (**3**)[7] (35 mg). Fraction 13 was eluted with *n*-hexane/EtOAc (5:1) and was further purified by recrystallization from $CHCl_3$ to give **1** (30 mg). Fraction 19 was further eluted with *n*-hexane/EtOAc (5:1) to give (5*S*,6*R*,7*R*,8*R*)-goniotriol (**7**)[1] (35 mg). The alkaloid layer was subjected to Si gel column chromatography and eluted with gradient mixtures of $CHCl_3$/MeOH; the eluates were combined into 10 fractions on the basis of TLC monitoring. Fraction 6 was further purified by recrystallization to give (+)-9-deoxygoniopypyrone (**9**)[9] (50 mg).

The same procedure applied to the stems was also used to process the fresh leaves (3.4 kg), which yielded $CHCl_3$ and aqueous extracts. The $CHCl_3$ extract was dried under vacuum to afford a residue (6.0 g), and the residue was subjected to Si gel column chromatography (2 kg, 33 × 11.5 cm) and eluted with increasingly polar *n*-hexane/$CHCl_3$/EtOAc/MeOH mixtures to yield 10 fractions. Fraction 4 was purified by repeated chromatography (Si gel, *n*-hexane/EtOAc) to afford cinnamic acid[17] (3.5 mg) and a mixture of β-sitosterol and stigmasterol[12] (20 mg). Fraction 5 was eluted with *n*-hexane/EtOAc (3:2) and was further purified by recrystallization from $CHCl_3$ to give **1** (3 mg). Fraction 7 was eluted with *n*-hexane/EtOAc (3:1) and was further purified by recrystallization from $CHCl_3$ to afford **2** (14 mg), goniothalamin epoxide (**3**)[7] (12 mg), and goniothalamin (**6**)[7,8] (20.6 mg). Fraction 8 was further purified on Si gel using *n*-hexane/EtOAc (1:1) to afford (5*S*,6*R*,7*R*,8*R*)-goniotriol (**7**)[1] (200 mg), goniodiol-7-monoacetate (**4**)[1] (960 mg), goniodiol-8-monoacetate (**5**)[1] (150 mg), and (5*S*,6*R*,7*S*,8*S*)-goniotriol (**8**)[12] (22 mg). Fraction 9 was rechromatographed on Si gel eluting with *n*-hexane/EtOAc (1:1) to afford liriodenine[13] (9.3 mg) and lysicamine[14] (3.3 mg). Fraction 10 was rechromatographed on Si gel eluting with $CHCl_3$/MeOH (5:1) to afford veratric acid[16] (4 mg), piperlactam C[10] (5.2 mg), and aristolactam FII[11] (4.2 mg).

8-Methoxygoniodiol (1): colorless prism crystals; mp 99−101 °C; $[\alpha]_{25}^{D}$ +24.2° (*c* 0.68, $CHCl_3$); IR (KBr) ν_{max} 3445, 2909, 1719, 1380, 1109, 1057, 757 cm^{-1}; CD ($CDCl_3$) [θ] +2.51 (251.1 nm), +2.24 (262.7 nm), +1.92 (272.4 nm); ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 248 $[M]^+$ (1), 230 $[M - H_2O]^+$ (1), 217 (1), 198 (4), 121 (100), 91 (49); HRFABMS *m*/*z* 249.1121 $[M]^+$ (calcd for $C_{14}H_{16}O_4$, 249.1116).

Crystal Data for 1. A colorless prism crystal of $C_{14}H_{16}O_4$ having approximate dimensions of 0.20 × 0.60 × 0.80 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7S diffractometer with graphite-monochromated Mo Kα radiation. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 20 carefully centered reflections in the range 9.27° < 2θ < 17.25°, corresponded to a C-centered monoclinic cell with dimensions a = 13291(2) Å, b = 9.831(4) Å, c = 10.031(4) Å, β = 99.31(2)°, V = 1293.5(7) Å^3. For Z = 4 and fw = 248.28, the calculated density is 1.27 g/cm^3, F(000) = 528.00, μ(Mo Kα) = 0.93 cm^{-1}.

8-Chlorogoniodiol (2): colorless plate crystals; mp 126−128 °C; $[\alpha]_{25}^{D}$ +13.7° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3387, 2918, 1714, 1480, 1380, 1251, 1104, 814, 742, 704 cm^{-1}; CD ($CDCl_3$) [θ] +2.35 (251.5 nm), +1.92 (263.8 nm), +1.67 (272.1 nm); ^{1}H

NMR and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 235.0527 $[M - H_2O]^+$ (calcd for $C_{13}H_{11}ClO_2$, 235.0533).

Crystal Data for 2. A colorless plate crystal of $C_{13}H_{13}ClO_3$ having approximate dimensions of 0.30 × 0.62 × 0.86 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7S diffractometer with graphite-monochromated Mo Kα radiation. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 17 carefully centered reflections in the range $8.72° < 2\theta < 13.44°$, corresponded to a primitive orthorhombic cell with dimensions $a = 7.863(4)$ Å, $b = 9.352(5)$ Å, $c = 33.355(7)$ Å, $V = 2453(1)$ Å^3. For $Z = 8$ and fw = 252.70, the calculated density is 1.37 g/cm^3, $F(000) = 1056.00$, μ(Mo Kα) = 3.04 cm^{-1}.

Cytotoxicity Assays. The cytotoxicity assays were carried out according to established protocols using the NUGC and HONE-1 cancer cell lines.[21,22]

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Supporting Information Available: The CD spectra of styrylpyrones **1**−**9** are available. This information is available free of charge via the Internet at http://pubs.acs.org.

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