

## Semisynthesis and Cytotoxicity of Styryl-Lactone Derivatives

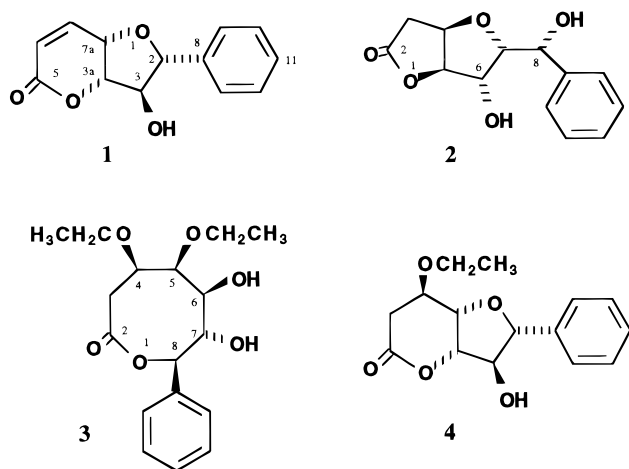
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The cytotoxicity and the cell-cycle action of altholactone (**1**), goniofufurone (**2**), and eight altholactone derivatives (**5**–**12**), were determined in vitro on L-1210 cells. Semisyntheses and structure–activity relationships of these compounds are described. The results of this study suggest that the cytotoxicity of altholactone (**1**), 11-nitro-altholactone (**8**), and 7-chloro-6,7-dihydroaltholactone (**10**) is due to the accumulation of the cells in the G2 + M phase of the cell cycle.

The styryl-lactones found abundantly in *Goniolthalamus* species (Annonaceae) are an interesting group of cytotoxic and antitumor agents.<sup>1</sup> Altholactone (**1**), a furano-pyrone first isolated in 1977<sup>2</sup> and recently identified as the major compound from the stem bark of *Goniolthalamus arvensis*,<sup>3</sup> is known to possess significant cytotoxicity against several tumor cell lines,<sup>4,5</sup> and stereoselective syntheses of **1** were carried out.<sup>6,7</sup> Goniofufurone (**2**), a known furano-furone also isolated from *G. arvensis*<sup>8</sup> and other furano or pyrano styryl-lactone derivatives, showed interesting biological activities.<sup>9,10</sup> The cytotoxic activities previously reported for eight-membered-ring  $\zeta$ -lactones (heptolides)<sup>11,12</sup> were recently explained by a noncompetitive mechanism of action as inhibitors of the mammalian respiratory chain complex I for almuheptolide A (**3**), a 8-phenyl-2-oxocanone isolated from *G. arvensis* and also synthesized from **1** via the 6,7-dihydro-7-ethoxy-altholactone (etharvensin, **4**).<sup>13</sup>



Herein we describe the semisynthesis of eight new styryl-lactone derivatives in the altholactone series (**5**–**12**). The cytotoxicity of these and the two natural compounds **1** and **2** are also reported.

### Results and Discussion

Altholactone (**1**), a 2-phenyl-3-hydroxy-6,7-dihydro-furano-pyrone, represents a good starting material because it is

available in large quantity from *G. arvensis*.<sup>3</sup> Treatment of **1** (previously 3-acetylated) with osmium tetroxide (OsO<sub>4</sub>) in water, and *N*-methylmorpholine *N*-oxide monohydrate (NMO) to regenerate the oxidizing agent,<sup>14</sup> afforded the corresponding *cis*-dihydroxylated derivative **5**. The 3-acetyl-6,7-diacetoxy-6,7-dihydro-altholactone (**6**) was obtained by treatment of **1** with NMO and OsO<sub>4</sub>, followed by acetic anhydride in dry pyridine. When a solution of **5** was treated with an excess of triethylamine and a precipitate obtained from Me<sub>3</sub>SiCl and Me<sub>2</sub>SO,<sup>15</sup> the 6,7-methylenedioxy derivative was afforded (**7**) (see Scheme 1).

The methylenedioxy moiety that was formed in **7** connects the diol of **5** but does not change the stereochemistry of their carbinol centers. A *cis*-configuration among the 6, 7, and 7a centers in these three last compounds (**5**–**7**) can be explained as in etharvensin (**4**)<sup>16</sup> by observation of their <sup>1</sup>H NMR coupling constants (*J*<sub>7a,7</sub> and *J*<sub>7,6</sub> between 3.5 and 4 Hz). On the other hand, when an organic solution of **1** was added to a mixture (1:1) of concentrated nitric acid and sulfuric acid, two nitro-altholactone derivatives, **8** (11-nitro-altholactone) and **9** (9,13-dinitro-altholactone), were obtained in similar yields. The mechanism of aromatic nitration involves an electrophilic attack of the nitronium ion at the ortho- or para-position in the monosubstituted aromatic ring of **1**.

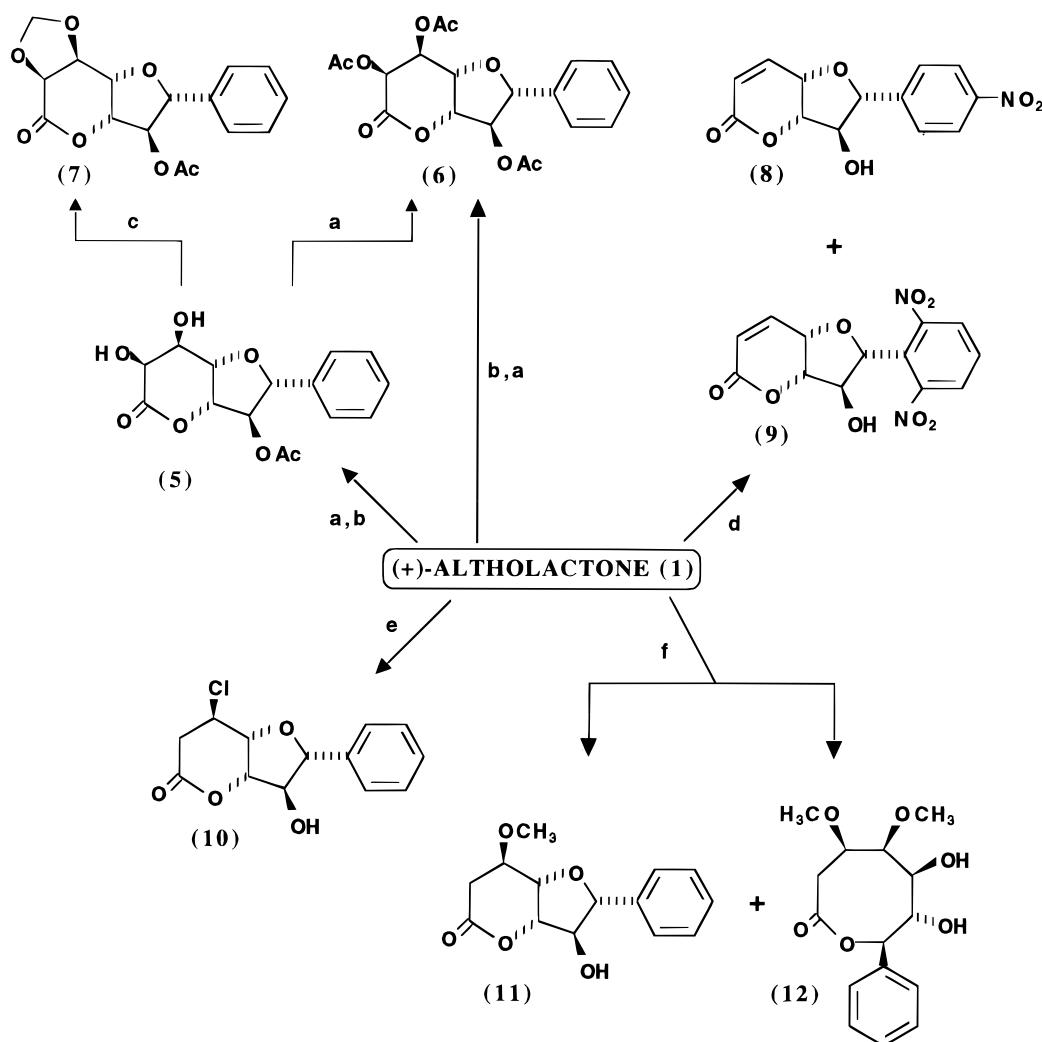
An original stereospecific chlorination at C-7 of **1** ( $\beta$  to the lactonic carbonyl) was carried out under acidic conditions using POCl<sub>3</sub> at room temperature. An EIMS fragment ion at *m/z* 232 [*M* – HCl]<sup>+</sup>, as well as a dd at  $\delta$  4.54 in <sup>1</sup>H NMR (H-7) with its characteristic coupling constant values corresponding to a *cis*-relationship between the neighbor protons (*J*<sub>6a,7</sub> = 3.7 Hz and *J*<sub>7a,7</sub> = 4 Hz) and a signal at  $\delta$  50.5 in <sup>13</sup>C NMR (C-7), were in agreement with a  $\beta$ -7-chloro-6,7-dihydro-altholactone (**10**).

A method for the preparation of both 7-alkoxylated-6,7-dihydro- $\delta$ -lactone (**11**) and 4,5-dialkoxylated eight-membered-ring  $\zeta$ -lactones with a heptolide skeleton (**12**), has been successfully achieved from **1**.<sup>13</sup> The enantiospecific syntheses of compounds **11** and **12** from **1** was carried out in a one-step reaction, using MeOH and concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of 7-methoxy-6,7-dihydro-altholactone (**11**) is postulated to be a direct Michael-type addition of MeOH from the less electron-dense position ( $\beta$  to the C=O).<sup>16</sup> Subsequent nucleophilic attack with a second MeOH molecule by a S<sub>N</sub>2 mechanism would lead to inversion of the configuration, followed by a concerted opening of the  $\alpha$ -pyrone and the tetrahydrofuran ring and a subsequent intramolecular eight-ring closure.<sup>13</sup>

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Scheme 1<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) Ac<sub>2</sub>O/Pyr, room temperature; (b) NMO/OsO<sub>4</sub> room temperature, followed by 2% NaHSO<sub>3</sub>, room temperature; (c) Me<sub>3</sub>SiCl/Me<sub>2</sub>SO, followed by (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N, room temperature; (d) HNO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub>, 0 °C; (e) POCl<sub>3</sub>/CH<sub>2</sub>Cl<sub>2</sub>, room temperature; (f) MeOH/H<sub>2</sub>SO<sub>4</sub>, reflux.

Table 1. Cytotoxic Activity of Styryl-Lactone Derivatives<sup>a</sup>

| compounds | IC <sub>50</sub> (μg/mL) |
|-----------|--------------------------|
| <b>1</b>  | 2.9                      |
| <b>8</b>  | 3.2                      |
| <b>9</b>  | 5.0                      |
| <b>10</b> | 3.7                      |
| <b>12</b> | 5.2                      |

<sup>a</sup> Inhibition of L-1210 cell proliferation measured by the MMT assay. IC<sub>50</sub> of compounds **2**, **5–7**, and **11** with IC<sub>50</sub> > 10 μg/mL.

In view of the biological interest focused on this class of compounds, a study of the cytotoxic activities of the known natural (**1** and **2**) and the new synthetic (**5–12**) styryl-lactone derivatives was carried out in vitro using L-1210 leukemia cells. Altholactone (**1**), some of its furano-pyrone derivatives (**8–10**) and the heptolide **12**, were more cytotoxic than the remaining derivatives (**2**, **5–7**, and **11**) inhibiting L-1210 cell proliferation (see Table 1). Structure-activity relationships in the styryl-lactone series indicate that the 6,7-double bond on the pyrone ring or the chlorination at 7 position is the more important structural requirement to increase the cytotoxicity. So, altholactone (**1**), the two nitro-derivatives on the aromatic ring (**8,9**), and the chloro dihydro-altholactone (**10**) were more cytotoxic than the furano-furone (**2**) or the 6,7-dihydro derivatives of altholactone, whether dihydroxylated (**5**), triacetylated (**6**), methylenedioxy (**7**), or methoxylated (**11**). Moreover, the

heptolide **12** shows a very important cytotoxicity, explained by the inhibition of the mammalian respiratory chain complex I.<sup>13</sup>

Perturbation of the cell cycle induced by compounds **1**, **8**, **9**, **10**, and **12** was studied using the same cell line. Altholactone (**1**), 11-nitro-altholactone (**8**), and 7-chloro-6,7-dihydro-altholactone (**10**) induced a partial accumulation (35–45%) of the cells in the G2 + M phase on the cell cycle. However, the 9,13-dinitro-altholactone (**9**) and the heptolide (**12**) showed a nonselective accumulation in the cell cycle.

## Experimental Section

**General Experimental Procedure.** Optical rotations were determined on a Perkin-Elmer 241 polarimeter. IR spectra (film) were run on a Perkin-Elmer 1750 FTIR spectrometer. MS were recorded with a VG Auto Spec Fisons spectrometer, and the liquid secondary ion mass spectrum (LSIMS) was obtained by fast ion bombardment. <sup>1</sup>H NMR (250, 300 and 400 MHz), <sup>13</sup>C NMR, and DEPT (62.5, 75 and 100 MHz) spectra were recorded on Bruker AC-250, Varian Unity-300, and Varian Unity-400 instruments. The signals of <sup>1</sup>H NMR of all the compounds were assigned by COSY 45. Si gel TLC plates were observed under UV light (254 nm) and after spraying with anisaldehyde in H<sub>2</sub>SO<sub>4</sub>.

**Plant Material.** *Goniiothalamus arvensis* Scheff. (Annonaceae) was collected in the National Park of Varirata, located

in the Central Province of Papua New Guinea. A voucher specimen was deposited in the herbarium of the University of Papua New Guinea.

**Extraction and Isolation.** Dried and powdered stem bark of *G. arvensis* (368 g) was macerated with MeOH at room temperature. The concentrated MeOH extract was partitioned between hexane and 50% aqueous MeOH. The aqueous MeOH extract was fractionated successively into CH<sub>2</sub>Cl<sub>2</sub> and EtOAc. The CH<sub>2</sub>Cl<sub>2</sub> extract (7.0 g) was applied by Si gel 60 H column chromatography to afford altholactone (**1**, 2.3 g) and goniofufurone (**2**, 165 mg).

**(+)-Altholactone (1):** C<sub>13</sub>H<sub>12</sub>O<sub>4</sub>; [α]<sub>D</sub> +174.2° (c 1.0, EtOH); EIMS *m/z* [M]<sup>+</sup> 232.<sup>3,17,18</sup>

**(+)-Goniofufurone (2):** C<sub>13</sub>H<sub>14</sub>O<sub>5</sub>; [α]<sub>D</sub> +12° (c 1.1, EtOH); mp 154–155 °C (EtOH–hexane); EIMS *m/z* [M]<sup>+</sup> 250.<sup>8,17,18</sup>

**(–)-3-Acetyl-6,7-dihydroxy-6,7-dihydro-altholactone (5).** Treatment of **1** (150 mg, 0.64 mmol) with dry pyridine (1 mL) and Ac<sub>2</sub>O (2 mL) afforded 3-acetyl-altholactone (170 mg; 0.62 mmol; 96%) after stirring for 1 h at room temperature. To a solution of 3-acetyl-altholactone (55 mg; 0.20 mmol) and *N*-methylmorpholine *N*-oxide monohydrate (NMO, 27.1 mg; 0.20 mmol) in 3 mL of Me<sub>2</sub>CO–H<sub>2</sub>O (2:1) was added a 4% solution of OsO<sub>4</sub> in H<sub>2</sub>O (0.3 mL; 0.05 mmol) dropwise. The reaction mixture was stirred at room temperature for 1.5 h, and then a 2% solution of NaHSO<sub>3</sub> in H<sub>2</sub>O (2 mL) was added. After being stirred for 30 min, the reaction mixture was extracted with EtOAc, which was washed with H<sub>2</sub>O and brine, and concentrated to dryness. The reaction residue was subjected to column chromatography on Si gel 60 H (eluted with CH<sub>2</sub>Cl<sub>2</sub>) to afford 46.5 mg of **5** (75% from 3-acetyl-altholactone): C<sub>15</sub>H<sub>16</sub>O<sub>7</sub>; [α]<sub>D</sub> –12.2° (c 1.8, EtOH); IR (dry film) ν<sub>max</sub> 3432 (OH), 2920, 1752 (C=O), 1373, 1235, 1117, 1047, 950, 914, 864, 763, 735, 700 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.38–7.30 (m, H-9 to H-13), 5.31 (dd, H-3), 5.08 (dd, H-3a), 4.88 (d, H-2), 4.62 and 4.60 (m, 2H, H-6 and H-7), 4.53 (m, H-7a), 2.13 (s, 3H, 3-OCOCH<sub>3</sub>); J<sub>2,3</sub> = 5.1 Hz; J<sub>3,3a</sub> = 1.4 Hz; J<sub>3a,7a</sub> = 3.9 Hz; J<sub>7a,7</sub> = 3.2 Hz; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 172.0 (C-5), 169.4 (3-OCOCH<sub>3</sub>), 136.9 (C-8), 128.8 (3 CH), 126.1 (2 CH), 85.0 (C-3a), 84.8 (C-2), 82.6 (C-3), 77.0 (C-7a), 68.1 (C-6 and C-7), 20.7 (3-OCOCH<sub>3</sub>); <sup>1</sup>H and <sup>13</sup>C spectra were unambiguously assigned by using 2D NMR techniques (COSY 45 and HMQC); EIMS *m/z* (%) [M]<sup>+</sup> 308 (5), 266 [M – COCH<sub>2</sub>]<sup>+</sup> (17), 248 [M – HOCOCH<sub>3</sub>]<sup>+</sup> (74), 232 [M – OCOCH<sub>3</sub> – OH]<sup>+</sup> (3), 215 [M – OCOCH<sub>3</sub> – 2 OH]<sup>+</sup> (1), 144 (100), 107 (78), 91 (49), 77 (32).

**3-Acetyl-6,7-diacetoxy-6,7-dihydro-altholactone (6).** To a solution of altholactone (**1**, 50 mg; 0.21 mmol) in 3 mL of Me<sub>2</sub>CO–H<sub>2</sub>O (2:1) was added NMO (27.1 mg; 0.20 mmol) and catalytic 4% solution of OsO<sub>4</sub> in H<sub>2</sub>O (0.3 mL; 0.05 mmol) dropwise, and the resulting solution was stirred at room temperature for 6 h. The reaction mixture was concentrated to dryness by blowing air over the solution, and the residue was dissolved in EtOAc and filtered through a plug of Si gel. The solution was concentrated to dryness, and the residue was dissolved in dry pyridine (1 mL) and Ac<sub>2</sub>O (2 mL). The resulting solution was stirred at room temperature for 4 h. The residue obtained was extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with H<sub>2</sub>O, and dried. The dry extract was subjected to column chromatography on Si gel 60 H (eluted with CH<sub>2</sub>Cl<sub>2</sub>–EtOAc 100:1) to give 39 mg of **6** (46%): C<sub>19</sub>H<sub>20</sub>O<sub>9</sub>; IR (dry film) ν<sub>max</sub> 2927, 1752 (C=O), 1372, 1218, 1047, 702 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 7.37–7.28 (m, H-9 to H-13), 5.90 (d, H-6), 5.78 (t, H-7), 5.35 (dd, H-3), 5.02 (dd, H-3a), 4.94 (d, H-2), 4.49 (t, H-7a), 2.17, 2.15 and 2.13 (3s, 9H, 3-, 6-, and 7-OCOCH<sub>3</sub>); J<sub>2,3</sub> = 4.7 Hz; J<sub>3,3a</sub> = 1.3 Hz; J<sub>3a,7a</sub> = 4.0 Hz; J<sub>7a,7</sub> = 3.8 Hz; J<sub>7,6</sub> = 3.4 Hz; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 62.5 MHz) δ 169.2 (3 C, 3-, 6-, and 7-OCOCH<sub>3</sub>), 164.3 (C-5), 136.4 (C-8), 128.9 (3 CH), 126.0 (2 CH), 85.4 (C-3a), 84.4 (C-2), 82.5 (C-3), 75.1 (C-7a), 68.2 and 65.8 (C-6 and C-7), 20.7, and 20.4 (3 C, 3-, 6-, and 7-OCOCH<sub>3</sub>); EIMS *m/z* (%) [MH]<sup>+</sup> 393 (5), 334 [M – OCOCH<sub>3</sub>]<sup>+</sup> (2), 274 [M – 2 OCOCH<sub>3</sub>]<sup>+</sup> (3), 215 [M – 3 OCOCH<sub>3</sub>]<sup>+</sup> (50), 185 (100), 155 (75), 105 (94), 91 (70), 77 (24).

**3-Acetyl-6,7-dihydro-6,7-methylenedioxy-altholactone (7).** To Me<sub>3</sub>SiCl (2 mL) was added Me<sub>2</sub>SO (2 mL) and dry CH<sub>2</sub>–

Cl<sub>2</sub> (2 mL), and the mixture was allowed to stand at room temperature for about 1 h until a white precipitate appeared. The excess of unreacted reagents was decanted, and the white precipitate was quickly washed with CH<sub>2</sub>Cl<sub>2</sub> (1 mL). A solution of **5** (25 mg; 0.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL), and an excess of (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N was added to this precipitate and stirred at room temperature for 15 h. When the reaction was complete as estimated by TLC, the mixture was washed using 1% NaHCO<sub>3</sub> (5 mL) and H<sub>2</sub>O (2 × 5 mL), and the CH<sub>2</sub>Cl<sub>2</sub> layer was evaporated in vacuo. The crude product was subjected to column chromatography on Si gel 60 H (eluted with CH<sub>2</sub>Cl<sub>2</sub>–EtOAc 60:40) to afford 14 mg of **7** (54%): C<sub>16</sub>H<sub>16</sub>O<sub>7</sub>; IR (dry film) ν<sub>max</sub> 2800, 1752 (C=O), 1238, 1155 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.35–7.30 (m, H-9 to H-13), 5.33 (dd, H-3), 5.11 and 5.06 (2d, 2 H, OCH<sub>2</sub>O), 4.99 (dd, H-3a), 4.91 (d, H-2), 4.87 (d, H-6), 4.68 (dd, H-7), 4.54 (t, H-7a), 2.14 (s, 3 H, OCOCH<sub>3</sub>); J<sub>2,3</sub> = 4.2 Hz; J<sub>3,3a</sub> = 1.2 Hz; J<sub>3a,7a</sub> = 3.9 Hz; J<sub>7a,7</sub> = 4.1 Hz; J<sub>7,6</sub> = 2.5 Hz; J<sub>OCH<sub>2</sub>O</sub> = 7.3 Hz; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 169.2 (3-COCH<sub>3</sub>), 167.0 (C-5), 136.9 (C-8), 128.9 (3 CH), 126.2 (2 CH), 94.8 (OCH<sub>2</sub>O), 85.8, 83.3 and 82.5 (3C, C-2, C-3 and C-3a), 74.6 and 72.4 (2C, C-6 and C-7), 20.8 (3-OCOCH<sub>3</sub>); EIMS *m/z* (%) [M]<sup>+</sup> 320 (3), 261 [M – OCOCH<sub>3</sub>]<sup>+</sup> (20), 233 (12), 145 (64), 133 (85).

**Semisyntheses of 8 and 9.** To 1.5 mL of concentrated HNO<sub>3</sub> was added dropwise at 0 °C concentrated H<sub>2</sub>SO<sub>4</sub> (0.5 mL). To a CH<sub>2</sub>Cl<sub>2</sub> solution (0.5 mL) of altholactone (**1**, 150 mg, 0.64 mmol) was added dropwise at 0 °C concentrated H<sub>2</sub>SO<sub>4</sub> (2.0 mL). Both solutions were mixed and stirred at room temperature for 30 min, then H<sub>2</sub>O was added and the reaction products extracted into CH<sub>2</sub>Cl<sub>2</sub>. The residue was subjected to column chromatography on Si gel 60 H (eluted with CH<sub>2</sub>Cl<sub>2</sub>–EtOAc 90:10) to afford 39 mg of **8** (22%) and 55 mg of **9** (27%).

**(+)-11-Nitro-altholactone (8):** C<sub>13</sub>H<sub>11</sub>NO<sub>6</sub>; [α]<sub>D</sub> +184° (c 0.7, EtOH); mp 178–180 °C (CHCl<sub>3</sub>–MeOH); IR (dry film) ν<sub>max</sub> 3356 (OH), 1714 (C=O), 1529 (NO<sub>2</sub>), 1339, 1243, 1089 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 8.16 (d, 2 H, H-10 and H-12), 7.51 (d, 2 H, H-9 and H-13), 7.05 (dd, H-7), 6.25 (d, H-6), 4.94 (dd, H-3a), 4.87 (d, H-2), 4.70 (t, H-7a), 4.38 (dd, H-3); J<sub>2,3</sub> = 5.3 Hz; J<sub>3,3a</sub> = 2.0 Hz; J<sub>3a,7a</sub> = 4.9 Hz; J<sub>7a,7</sub> = 5.0 Hz; J<sub>7,6</sub> = 10.0 Hz; J<sub>9,10</sub> = J<sub>12,13</sub> = 8.7 Hz; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 161.2 (C-5), 147.6 (C-8), 145.7 (C-11), 140.1 (C-7), 126.4 (2 CH, C-9 and C-13), 123.9 (C-6), 123.8 (2 CH, C-10 and C-12), 86.3 (C-3a), 84.8 (C-2), 83.5 (C-3), 68.4 (C-7a); LSIMS *m/z* 300 [M + Na]<sup>+</sup>, 278 [MH]<sup>+</sup>; EIMS *m/z* (%) [M]<sup>+</sup> 277 (7), 232 [MH – NO<sub>2</sub>]<sup>+</sup> (10), 213 [M – NO<sub>2</sub> – H<sub>2</sub>O]<sup>+</sup> (20), 122 (16), 97 (100).

**(+)-9,13-Dinitro-altholactone (9):** C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>8</sub>; [α]<sub>D</sub> +155° (c 0.8, EtOH); mp 103–106 °C (CHCl<sub>3</sub>–MeOH); IR (dry film) ν<sub>max</sub> 3382 (OH), 1718 (C=O), 1529 (NO<sub>2</sub>), 1340, 1244, 1102, 1052 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.23 (d, 2 H, H-10 and H-12), 7.53 (d, H-11), 7.07 (dd, H-7), 6.35 (d, H-6), 5.46 (dd, H-3a), 5.12 (d, H-2), 5.08 (br t, H-3), 4.63 (dd, H-7a); J<sub>2,3</sub> = 3.7 Hz; J<sub>3,3a</sub> ≈ 1.0 Hz; J<sub>3a,7a</sub> = 3.9 Hz; J<sub>7a,7</sub> = 5.4 Hz; J<sub>7,6</sub> = 9.8 Hz; J<sub>10,11</sub> = J<sub>11,12</sub> = 9.0 Hz; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 159.5 (C-5), 148.1 (C-8), 143.6 (2 C, C-9 and C-13), 138.1 (C-7), 126.9 (2 CH, C-10 and C-12), 125.2 (C-11), 124.1 (C-6), 90.8 (C-3a), 83.0 (C-2), 82.5 (C-3), 69.5 (C-7a); LSIMS *m/z* 345 [M + Na]<sup>+</sup>, 323 [MH]<sup>+</sup>; EIMS *m/z* (%) [MH]<sup>+</sup> 323 (10), 277 [MH – NO<sub>2</sub>]<sup>+</sup> (40), 231 [MH – 2 NO<sub>2</sub>]<sup>+</sup> (19), 213 [M – OH – 2 NO<sub>2</sub>]<sup>+</sup> (10), 167 (87), 165 (100).

**(+)-6,7-Dihydro-7-chloro-altholactone (10).** To a dry CH<sub>2</sub>Cl<sub>2</sub> solution (10 mL) of altholactone (**1**, 50 mg, 0.21 mmol) was added POCl<sub>3</sub> (1.0 mL; 0.5 mmol) dropwise. After stirring for 4 h at room temperature, the reagents were removed under reduced pressure, then H<sub>2</sub>O was added and the reaction mixture extracted into CH<sub>2</sub>Cl<sub>2</sub>. The organic solution after usual workup was purified by column chromatography on Si gel 60 H (eluted with CH<sub>2</sub>Cl<sub>2</sub>–EtOAc 90:10), to afford 30 mg of **10** (52%): C<sub>13</sub>H<sub>13</sub>O<sub>4</sub>Cl; [α]<sub>D</sub> +22.3° (c 2.8, EtOH); IR (dry film) ν<sub>max</sub> 3421 (OH), 3035, 2920, 1733 (C=O), 1386, 1055, 762, 700 cm<sup>–1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.37–7.28 (m, H-9 to H-13), 5.04 (dd, H-3a), 4.78 (d, H-2), 4.54 (dd, H-7), 4.51 (t, H-7a), 4.34 (dd, H-3), 3.21 (dd, H-6b), 2.93 (dd, H-6a); J<sub>2,3</sub> = 5.6 Hz; J<sub>3,3a</sub> = 1.4 Hz; J<sub>3a,7a</sub> = 4.0 Hz; J<sub>7a,7</sub> = 4.1 Hz; J<sub>7,6b</sub> = J<sub>7,6a</sub> = 3.7

Hz;  $J_{6a,6b} = 17.5$  Hz;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  167.1 (C-5), 137.7 (C-8), 128.8 (2 CH), 128.5 (CH), 125.9 (2 CH), 86.9 (C-3a), 86.8 (C-2), 83.4 (C-3), 75.4 (C-7a), 50.6 (C-7), 34.9 (6- $\text{CH}_2$ );  $^1\text{H}$  and  $^{13}\text{C}$  spectra were unambiguously assigned by using 2D NMR techniques (COSY 45 and HMQC); EIMS  $m/z$  (%)  $[\text{M}]^+$  268.5 (14), 251  $[\text{M} - \text{OH}]^+$  (15), 232  $[\text{M} - \text{HCl}]^+$  (64), 215  $[\text{M} - \text{OH} - \text{HCl}]^+$  (5), 107 (83), 97 (100), 91 (62), 77 (60).

(-)-**6,7-Dihydro-7-methoxy-altholactone (11)**:  $\text{C}_{14}\text{H}_{16}\text{O}_5$ ;  $[\alpha]_{\text{D}} -69.2^\circ$  (c 1.3, EtOH); EIMS  $m/z$   $[\text{M}]^+$  264; proposed according to literature.<sup>13</sup>

(+)-**4,5-Methoxy-6,7-hydroxy-8-phenyl-oxocanone (12)**:  $\text{C}_{15}\text{H}_{20}\text{O}_6$ ;  $[\alpha]_{\text{D}} +15^\circ$  (c 0.8, EtOH); LSIMS  $m/z$   $[\text{MH}]^+$  297; proposed according to literature.<sup>13</sup>

**Cytotoxicity and Cell Culture.** L-1210 cells were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 units/mL of penicillin, 50  $\mu\text{g}$ /mL of streptomycin, and 10 mM of Hepes buffer (pH = 7.4). Cells were exposed to graded concentrations of drug for 48 h. Cytotoxicity was measured by the microculture tetrazolium assay.<sup>19</sup> Results are expressed as  $\text{IC}_{50}$ , the concentration needed to reduce by 50% the optical density of treated cells with respect to the optical density of untreated controls.

For the cell-cycle analysis, L-1210 cells were incubated for 21 h at  $37^\circ\text{C}$  with various concentrations of drugs. Cells were then fixed by 70% EtOH (v/v), washed, and incubated with PBS containing 100  $\mu\text{g}$ /mL RNase and 25  $\mu\text{g}$ /mL propidium iodide for 30 min at  $20^\circ\text{C}$ . Results are expressed as percentage of accumulated cells in the G2 + M phase after 21 h compared with the control.

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