

SYNTHESIS AND CYTOTOXIC ACTIVITY OF ISOACRONYCINE AND ITS DERIVATIVES

Prokopios Magiatis,^a Sofia Mitaku,^{a*} Alain Pierré,^b and Ghanem Atassi^b

^aLaboratory of Pharmacognosy, University of Athens, Panepistimiopolis, Zografou, GR-15771 Athens, Greece. E-mail: mitakou@pharm.uoa.gr

^bInstitut de Reserches SERVIER, 11 rue des Moulineaux, 92150 Suresnes, France

Abstract – Condensation of *N*-methyl-1,3-dihydroxyacridone with 3-methyl-2-butenal led selectively to norisoacronycine, which upon methylation gave isoacronycine. Functionalization of the 1,2 double bond of isoacronycine led to derivatives with reduced cytotoxicity compared with the corresponding ones deriving from acronycine. Two very interesting exceptions were 1-hydroxy-1,2-dihydroisoacronycine (**14**) and its acetate (**16**), which showed strong induction of apoptosis.

Isoacronycine (**1**), is the linear isomer of the antitumor alkaloid acronycine (**2**).¹⁻⁴ Although isoacronycine has never been isolated as a natural product, there are six natural alkaloids that possess the pyrano[3,2-*b*]acridinone skeleton.⁵ In contrast to acronycine and its derivatives which have been systematically studied, neither isoacronycine nor any of its natural analogues have ever been studied for their biological activity. Isoacronycine has been many times obtained as a byproduct during the syntheses of acronycine.⁶ However, there are two selective synthetic approaches, one by Greco *et al.*⁷ and one by Reisch *et al.*⁸ We report herein a modified selective synthesis of isoacronycine with less steps and increased overall yield. In addition, eleven new derivatives of isoacronycine have been synthesized and tested for their cytotoxic activity, in order to investigate the role of the type of the pyrano fusion to the cytotoxicity of the pyranoacridones.

Both Greco *et al.*⁷ and Reisch *et al.*⁸ had used *N*-methyl-1,3-dihydroxyacridone (**3**) as the starting material for the synthesis of isoacronycine. According to Greco *et al.* the introduction of the linear pyrano ring was achieved by condensation with malic acid, followed by treatment with MeLi. Reisch *et al.* performed the condensation with methylbutenol to the 2-iodo derivative of **3**. The iodine atom at position 2 was supposed to direct the cyclization towards the linear isomer.

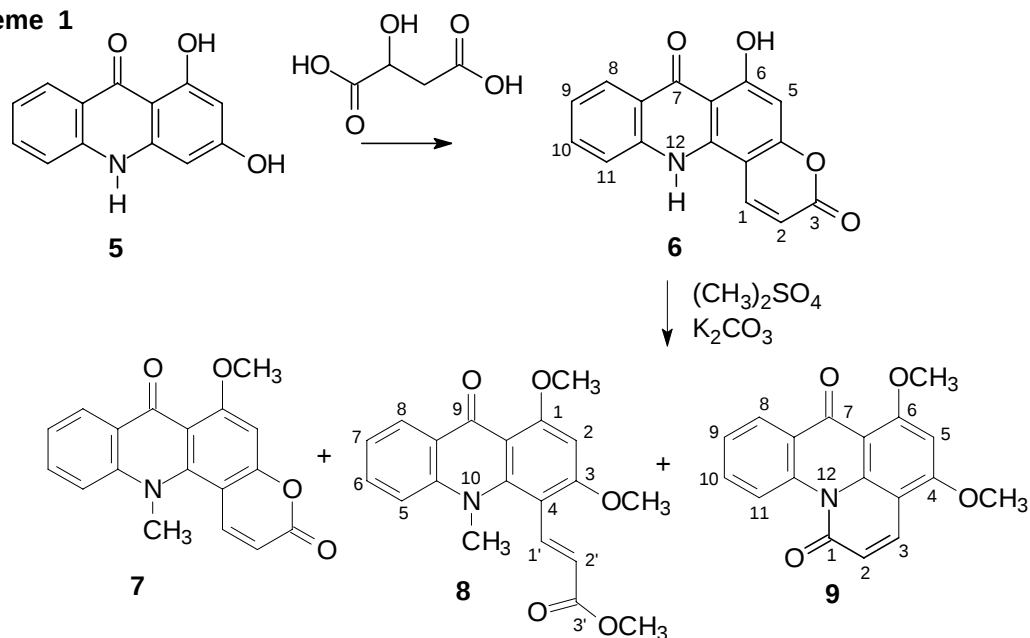
Based on a previous successful use of 3-methyl-2-butenal in the preparation of linear pyranoquinones,⁹ we envisaged a similar approach in the synthesis of isoacronycine. Indeed, condensation of **3**¹⁰ with 3-methyl-2-butenal in pyridine led selectively and in 45% yield to nor-isoacronycine (**4**). The rest starting material (55%) was unchanged and it could be recycled, increasing the total yield. Only traces of the angular product of condensation were prepared (<0.5%).

Interestingly, when the above-described reaction was performed using 1,3-dihydroxyacridone (**5**) as the starting material, the result was a mixture of nor-acronycine and nor-isoacronycine (9:1) with total yield again 45%. This reaction proved that the most important factor that controls the type of fusion during the condensation is the presence or not of the *N*-methyl group. Obviously, the iodination of **3** according the synthesis of Reisch is not necessary.

The crucial role of the *N*-methyl group was also confirmed when the condensation with malic acid, described by Greco, was performed using 1,3-dihydroxyacridone (**5**) as the starting material (Scheme 1). In this case, only the angular product (**6**) was obtained. Methylation of this product led partially to the permethylated product (**7**) and additionally to an opened product (**8**) and an interesting rearranged product (**9**).

In conclusion, the proposed synthesis of **1**, using 3-methyl-2-butenal is more simple and with increased yield.

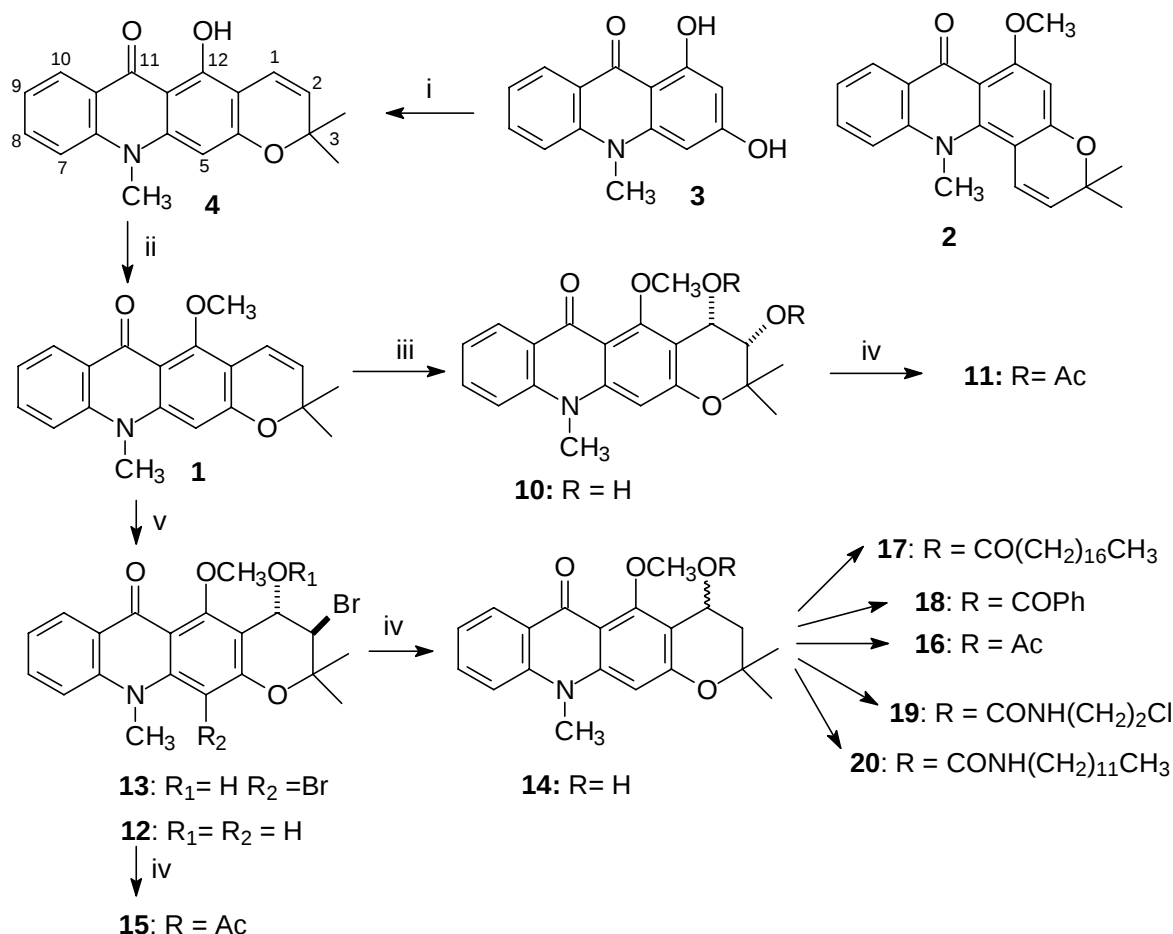
Scheme 1



As far as it concerns the derivatization of **1** (Scheme 2), the diol (**10**) has been obtained by catalytic osmium oxidation of **1** using *N*-methylmorpholine-*N*-oxide to regenerate the oxidizing agent. Treatment of *cis*-diol (**10**) with excess Ac_2O in pyridine afforded the corresponding diester (**11**). The bromohydrins (**12**) and (**13**) were obtained by treatment of **1** with NBS in aqueous THF solution.¹¹ Compound (**12**) was then smoothly debrominated with tributyltin hydride¹¹ to give **14**. Treatment of bromohydrin (**12**) and

alcohol (**14**) in a similar way like (**10**) afforded the corresponding esters (**15**) and (**16**). Use of the appropriate acyl chloride, anhydride or isocyanate led to the corresponding aliphatic (**17**), aromatic (**18**) or carbamidic ester (**19**, **20**) of **14**.

Scheme 2^a



^aKey (i) 3-methyl-2-butenal, Py, 130°C; (ii) (CH₃)₂SO₄, NaH, DMF, 55°C; (iii) OsO₄, *N*-methylmorpholine-*N*-oxide, *t*-BuOH, THF, H₂O, rt; (iv) Ac₂O, Py, rt; (v) NBS, THF, H₂O, 0°C; (viii) Bu₃SnH, AIBN, toluene, 110°C.

Isoacronycine and all its synthesized derivatives were tested against L-1210 leukemia cells. Almost all the tested compounds (**1**, **4**, **6-13**, **15**, **17-20**) were less active than the corresponding derivatives of acronycine, with IC₅₀ >10 μM, revealing the crucial role of the angular fusion of the pyrano ring. Interestingly, two compounds (**14**) and (**16**) were very active with IC₅₀ = 3.6 and 1.5 μM respectively. The cell cycle analysis for these two compounds showed that the distribution of cells in the different phases of the cell cycle (G1, S, G2/M) was not modified, but a population with a lower DNA content appeared (sub G1), indicative of apoptosis. When the cells were treated with 10 μM of **16** or 25 μM of **14**, as much as 73% and 39% of cells respectively, were in sub G1. It should be noted that the corresponding derivatives in the acronycine series are very unstable and they have never been tested for their pharmacological activities.

EXPERIMENTAL

Spectra were recorded on the following apparatus: IR, Perkin-Elmer Paragon 500. MS, Nermag R10-10C in desorption-chemical ionization, using NH_3 as reagent gas. NMR, Bruker AC 200, ^1H -NMR (200 MHz), ^{13}C -NMR (50 MHz) and a Bruker DRX400, ^1H -NMR (400 MHz). Chemical shifts are given in δ with TMS as an internal standard. Coupling constants (J) are given in Hz. The signals of ^1H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^1H - ^1H -COSY, ^{13}C - ^1H HMQC and HMBC. These 2D experiments were performed using standard Bruker microprograms. Column chromatographies were conducted using flash silica gel 60 Merck (40-63 μm), with an overpressure of 300 mbars. All new compounds gave satisfactory combustion analyses (C, H, N, within $\pm 0.4\%$ of calculated values).

Norisoacronycine (4). To a solution of *N*-methyl-1,3-dihydroxyacridone (**3**) (200 mg, 0.83 mmol) in dry pyridine (13 mL) was added 3-methyl-2-butenal (0.36 mL, 3.8 mmol) and the reaction mixture was stirred for 1.5 h at 115 $^\circ\text{C}$. Then the reagents were removed under reduced pressure (using a high vacuum pump) and the residue was submitted to flash chromatography on silica gel with cyclohexane: EtOAc (95: 5) to give **4** (115 mg, 45%). mp 200 $^\circ\text{C}$ (EtOAc). IR (CHCl_3) ν_{max} 3590, 1641, 1618, 1594, 1560, 1325 cm^{-1} . ^1H -NMR (CDCl_3 , 400 MHz) δ : 15.12 (1H, s, 12-OH), 8.40 (1H, dd, $J = 8, 1.5$ Hz, H-10), 7.67 (1H, td, $J = 8, 1.5$ Hz, H-8), 7.42 (1H, d, $J = 8$ Hz, H-7), 7.24 (1H, t, $J = 8$ Hz, H-9), 6.76 (1H, d, $J = 10$ Hz, H-1), 6.26 (1H, s, H-5), 5.56 (1H, d, $J = 10$ Hz, H-2), 3.74 (3H, s, NCH_3), 1.47 (6H, s, $2\times\text{CH}_3$). ^{13}C -NMR (CDCl_3 , 50 MHz) δ : 181.32 (C-11), 160.83 (C-12), 160.66 (C-4a), 145.03 (C-5a), 142.67 (C-6a), 134.61 (C-8), 127.52 (C-2), 127.24 (C-10), 123.85 (C-10a), 121.85 (C-9), 116.33 (C-1), 115.33 (C-7), 105.97 (C-11a), 103.14 (C-12a), 91.79 (C-5), 78.75 (C-3), 34.52 (NCH_3), 29.13 ($2\times\text{CH}_3$). MS-DCI m/z : 308 ($\text{M}+\text{H}$) $^+$.

Isoacronycine (1). To a solution of **4** (332 mg, 1.08 mmol) in dry DMF (5 mL) was added 60% NaH (110 mg, 2.7 mmol) and the reaction mixture was stirred under Ar for 30 min. Then dimethyl sulfate (0.4 mL, 4.2 mmol) was added and the stirring was continued for 4 h at 60 $^\circ\text{C}$. The reagents were removed under reduced pressure (using a high vacuum pump), the residue was extracted with $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ and the organic layer was collected and evaporated. The remaining solid was submitted to flash chromatography on silica gel with cyclohexane: EtOAc (95: 5 to 80: 20) to give **1** (180 mg, 52%). mp 161 $^\circ\text{C}$ (cyclohexane-EtOAc). IR (CHCl_3) ν_{max} 1632, 1602, 1552, 1493, 1307, 1134 cm^{-1} . ^1H -NMR (CDCl_3 , 400 MHz) δ : 8.50 (1H, dd, $J = 8, 1.5$ Hz, H-10), 7.65 (1H, td, $J = 8, 1.5$ Hz, H-8), 7.41 (1H, d, $J = 8$ Hz, H-7), 7.26 (1H, t, $J = 8$ Hz, H-9), 6.82 (1H, d, $J = 10$ Hz, H-1), 6.73 (1H, s, H-5), 5.71 (1H, d, $J = 10$ Hz, H-2), 3.99 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 1.52 (6H, s, $2\times\text{CH}_3$). ^{13}C -NMR (CDCl_3 , 50 MHz) δ : 177.75 (C-11), 163.51 (C-12), 159.73 (C-4a), 147.25 (C-5a), 144.96 (C-6a), 132.95 (C-8), 127.53 (C-10), 125.85 (C-

10a), 123.30 (C-2), 122.22 (C-9), 122.17 (C-1), 116.23 (C-7), 110.89 (C-11a), 103.32 (C-12a), 94.75 (C-5), 76.56 (C-3), 56.46 (OCH₃), 44.38 (NCH₃), 26.93 (2xCH₃). MS-DCI *m/z*: 322 (M+H)⁺.

cis-1,2-Dihydroxy-1,2-dihydroisoacronycine (10). To a solution of **1** (50 mg, 0.16 mmol) in 10 mL *t*-BuOH-THF-H₂O (10:3:1 v/v/v) was added a solution of OsO₄ 2.5% (w/v) in *t*-BuOH (0.3 mL) and 60 mg (0.39 mmol) of 4-methylmorpholine-*N*-oxide. The reaction mixture was stirred for 48 h at rt. Then NaHSO₃ (sat.) was added and the mixture was stirred for 1 h. Then the reaction mixture was extracted with CH₂Cl₂-H₂O and the organic layer was collected. The solvent was removed under reduced pressure and compound (**10**) was purified by flash chromatography on silica gel with CH₂Cl₂: MeOH (99: 1 to 96: 4) (31 mg, 55%). mp 249 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 3522, 1635, 1604, 1557, 1320 cm⁻¹. ¹H-NMR (DMSO, D₂O, 400 MHz) δ : 8.22 (1H, d, *J* = 8 Hz, H-10), 7.71 (1H, t, *J* = 8 Hz, H-8), 7.66 (1H, d, *J* = 8 Hz, H-7), 7.24 (1H, t, *J* = 8 Hz, H-9), 6.73 (1H, s, H-5), 4.84 (1H, d, *J* = 4.5 Hz, H-1), 3.84 (3H, s, OCH₃), 3.72 (3H, s, NCH₃), 3.58 (1H, d, *J* = 4.5 Hz, H-2), 1.36 (3H, s, CH₃), 1.35 (3H, s, CH₃). ¹³C-NMR (DMSO, 50 MHz) δ : 175.98 (C-11), 162.87 (C-12), 158.34 (C-4a), 146.74 (C-5a), 142.71 (C-6a), 134.14 (C-8), 127.58 (C-10), 123.55 (C-10a), 122.04 (C-9), 116.49 (C-7), 113.97 (C-12a), 110.95 (C-11a), 97.84 (C-5), 79.69 (C-3), 72.63 (C-2), 63.05 (OCH₃), 61.54 (C-1), 35.33 (NCH₃), 27.77 (CH₃), 22.22 (CH₃). MS-DCI *m/z*: 356 (M+H)⁺. Anal. Calcd for C₂₀H₂₁NO₅: C: 67.59, H: 5.96, N: 3.94. Found: C: 67.69, H: 5.98, N: 3.88.

cis-1,2-Diacetoxy-1,2-dihydroisoacronycine (11). To a solution of **10** (15 mg, 0.04 mmol) in dry pyridine (1.5 mL) was added Ac₂O (1.5 mL, 15 mmol). The reaction mixture was stirred for 24 h at rt and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was crystallized with cyclohexane-EtOAc to give compound (**11**) (16 mg, 87%). mp 292 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1744, 1635, 1604, 1557, 1319, 1242 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.44 (1H, dd, *J* = 8, 1.5 Hz, H-10), 7.64 (1H, td, *J* = 8, 1.5 Hz, H-8), 7.39 (1H, d, *J* = 8 Hz, H-7), 7.23 (1H, t, *J* = 8 Hz, H-9), 6.66 (1H, s, H-5), 6.44 (1H, d, *J* = 5 Hz, H-1), 5.23 (1H, d, *J* = 5 Hz, H-2), 3.93 (3H, s, OCH₃), 3.75 (3H, s, NCH₃), 2.09 (1H, s, COCH₃), 2.07 (1H, s, COCH₃), 1.45 (3H, s, CH₃), 1.42 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.25 (C-11), 169.76 (COCH₃), 169.70 (COCH₃), 162.89 (C-12), 157.86 (C-4a), 146.34 (C-5a), 141.58 (C-6a), 132.85 (C-8), 127.69 (C-10), 123.85 (C-10a), 120.97 (C-9), 113.98 (C-7), 111.14 (C-11a), 107.36 (C-12a), 97.44 (C-5), 76.96 (C-3), 71.13 (C-2), 62.23 (OCH₃), 60.74 (C-1), 34.54 (NCH₃), 26.42 (CH₃), 21.66 (CH₃), 20.78 (2xC₂H₅OCH₃). MS-DCI *m/z*: 440 (M+H)⁺. Anal. Calcd for C₂₄H₂₅NO₇: C: 65.59, H: 5.73, N: 3.19. Found: C: 67.50, H: 5.88, N: 3.24.

trans-2-Bromo-1-hydroxy-1,2-dihydroisoacronycine (12) and trans-2,5-dibromo-1-hydroxy-1,2-dihydroisoacronycine (13). To a solution of **1** (110 mg, 0.34 mmol) in THF (5 mL) and H₂O (5 mL) was added *N*-bromosuccinimide (62 mg, 0.35 mmol). The reaction mixture was stirred for 1 h at 0 °C and then

the reaction mixture was extracted with NaCl (sat.)/CH₂Cl₂ and the organic layer was collected. The solvent was removed under reduced pressure and the residue was submitted to flash chromatography on silica gel with CH₂Cl₂: MeOH (99: 5 to 98: 2) to give **12** (100 mg, 70%) and **13** (15 mg, 9%).

12: mp 246 °C (EtOAc). IR (CHCl₃) $\nu_{\max}$ 3566, 1634, 1605, 1557, 1318 cm⁻¹. ¹H-NMR (DMSO, 200 MHz) δ : 8.24 (1H, d, J = 8 Hz, H-10), 7.70 (2H, br s, H-8,7), 7.24 (1H, t, J = 8 Hz, H-9), 6.85 (1H, s, H-5), 6.33 (1H, d, J = 3.5 Hz, 1-OH), 5.17 (1H, br s, H-1), 4.60 (1H, d, J = 2 Hz, H-2), 3.87 (3H, s, OCH₃), 3.77 (3H, s, NCH₃), 1.61 (3H, s, CH₃), 1.53 (3H, s, CH₃). ¹³C-NMR (DMSO, 50 MHz) δ : 176.08 (C-11), 163.47 (C-12), 158.14 (C-4a), 145.93 (C-5a), 142.99 (C-6a), 134.25 (C-8), 127.48 (C-10), 123.48 (C-10a), 121.55 (C-9), 116.58 (C-7), 112.31 (C-11a), 110.78 (C-12a), 98.41 (C-5), 77.49 (C-3), 66.38 (C-1), 63.15 (OCH₃), 60.90 (C-2), 35.46 (NCH₃), 29.82 (CH₃), 25.96 (CH₃). MS-DCI m/z : 419, 417 (M+H)⁺. Anal. Calcd for C₂₀H₂₀NO₄Br: C: 57.43, H: 4.82, N: 3.35, Br: 19.10. Found: C: 57.39, H: 4.86, N: 3.48, Br: 19.22.

13: mp 258 °C (EtOAc). IR (CHCl₃) $\nu_{\max}$ 3560, 1634, 1603, 1557, 1318 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.29 (1H, dd, J = 8, 1.5 Hz, H-10), 7.65 (1H, td, J = 8, 1.5 Hz, H-8), 7.39 (1H, d, J = 8 Hz, H-7), 7.25 (1H, t, J = 8 Hz, H-9), 5.27 (1H, d, J = 6.5 Hz, H-1), 4.25 (1H, d, J = 6.5 Hz, H-2), 4.06 (3H, s, OCH₃), 3.91 (3H, s, NCH₃), 1.67 (3H, s, CH₃), 1.53 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.74 (C-11), 159.61 (C-12), 154.61 (C-4a), 148.36 (C-5a), 145.78 (C-6a), 133.64 (C-8), 130.89 (C-11a), 128.80 (C-5), 126.79 (C-10), 124.45 (C-10a), 122.26 (C-9), 116.79 (C-7), 113.23 (C-12a), 80.46 (C-3), 68.53 (C-1), 62.68 (OCH₃), 57.29 (C-2), 45.41 (NCH₃), 26.94 (CH₃), 23.15 (CH₃). MS-DCI m/z : 499, 497, 495 (M+H)⁺. Anal. Calcd for C₂₀H₁₉NO₄Br₂: C: 48.32, H: 3.85, N: 2.82, Br: 32.14. Found: C: 48.39, H: 3.87, N: 2.68, Br: 32.21.

trans-1-Acetoxy-2-bromo-1,2-dihydroisoacronycine (15). Treatment of **12** (15 mg, 0.04 mmol) in conditions essentially similar to those described for the preparation of **11** afforded compound (**15**) (15 mg, 90%). mp 253 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1732, 1634, 1605, 1557, 1318 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.45 (1H, dd, J = 8, 1.5 Hz, H-10), 7.64 (1H, td, J = 8, 1.5 Hz, H-8), 7.39 (1H, d, J = 8 Hz, H-7), 7.24 (1H, t, J = 8 Hz, H-9), 6.72 (1H, s, H-5), 6.44 (1H, d, J = 3.3 Hz, H-1), 4.31 (1H, d, J = 5 Hz, H-2), 3.95 (3H, s, OCH₃), 3.77 (3H, s, NCH₃), 2.11 (1H, s, COCH₃), 1.61 (3H, s, CH₃), 1.58 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.57 (C-11), 169.78 (COCH₃), 163.15 (C-12), 157.36 (C-4a), 146.42 (C-5a), 141.78 (C-6a), 133.17 (C-8), 127.70 (C-10), 123.89 (C-10a), 121.24 (C-9), 114.12 (C-7), 111.63 (C-11a), 105.83 (C-12a), 97.55 (C-5), 77.00 (C-3), 67.02 (C-1), 62.21 (OCH₃), 54.93 (C-2), 34.38 (NCH₃), 27.76 (CH₃), 25.11 (CH₃), 20.80 (COCH₃). MS-DCI m/z : 461, 459 (M+H)⁺. Anal. Calcd for C₂₂H₂₂NO₅Br: C: 57.40, H: 4.82, N: 3.04, Br: 17.36. Found: C: 57.46, H: 4.82, N: 3.08, Br: 17.20.

1-Hydroxy-1,2-dihydroisoacronycine (14). Compound (**12**) (110 mg, 0.26 mmol) was dissolved in anhydrous toluene (10 mL) and the solution was refluxed for 15 min under argon. Then were added AIBN (10 mg) and after 5 min a solution of tributyltin hydride (0.5 mL in 4 mL of toluene) with a rate of 0.5 mL/5 min. The reaction mixture was refluxed for 1 h. Then the solvent was evaporated and the residue was purified by flash chromatography on silica gel with CH₂Cl₂: MeOH (99.6: 0.4 to 98.5: 1.5) to give compound (**14**) (51 mg, 58%). mp 158 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 3530, 1633, 1604, 1553, 1320, 1133 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.42 (1H, dd, J = 8, 1 Hz, H-10), 7.58 (1H, td, J = 8, 1 Hz, H-8), 7.34 (1H, d, J = 8 Hz, H-7), 7.17 (1H, t, J = 8 Hz, H-9), 6.61 (1H, s, H-5), 5.11 (1H, t, J = 5.5 Hz, H-1), 4.03 (3H, s, OCH₃), 3.69 (3H, s, NCH₃), 2.11 (1H, dd, J = 14, 5.5 Hz, H-2a), 2.06 (1H, dd, J = 14, 5.5 Hz, H-2b), 1.44 (3H, s, CH₃), 1.39 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.84 (C-11), 161.87 (C-12), 158.54 (C-4a), 146.28 (C-5a), 142.32 (C-6a), 133.59 (C-8), 127.98 (C-10), 124.83 (C-10a), 121.53 (C-9), 114.46 (C-7), 113.63 (C-12a), 111.14 (C-11a), 98.25 (C-5), 76.77 (C-3), 62.85 (OCH₃), 60.35 (C-1), 40.54 (C-2), 34.82 (NCH₃), 28.22 (CH₃), 27.50 (CH₃). MS-DCI m/z : 340 (M+H)⁺. Anal. Calcd for C₂₀H₂₁NO₄: C: 70.78, H: 6.24, N: 4.13. Found: C: 70.69, H: 6.29, N: 4.14.

1-Acetoxy-1,2-dihydroisoacronycine (16). Treatment of **14** (15 mg, 0.04 mmol) in conditions essentially similar to those described for the preparation of **11** afforded compound (**14**) (16 mg, 89%). mp 181 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1733, 1630, 1602, 1555, 1320, 1241 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.45 (1H, dd, J = 8, 1.5 Hz, H-10), 7.63 (1H, td, J = 8, 1.5 Hz, H-8), 7.39 (1H, d, J = 8 Hz, H-7), 7.22 (1H, t, J = 8 Hz, H-9), 6.64 (1H, s, H-5), 6.24 (1H, dd, J = 5, 3 Hz, H-1), 3.94 (3H, s, OCH₃), 3.74 (3H, s, NCH₃), 2.18 (1H, dd, J = 15, 3 Hz, H-2a), 2.11 (1H, dd, J = 15, 5 Hz, H-2b), 2.07 (1H, s, COCH₃), 1.48 (3H, s, CH₃), 1.44 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 177.12 (C-11), 170.96 (COCH₃), 163.25 (C-12), 159.62 (C-4a), 146.86 (C-5a), 142.54 (C-6a), 133.69 (C-8), 128.13 (C-10), 124.43 (C-10a), 121.55 (C-9), 114.76 (C-7), 111.67 (C-11a), 108.79 (C-12a), 98.08 (C-5), 75.83 (C-3), 62.86 (OCH₃), 61.99 (C-1), 39.06 (C-2), 34.95 (NCH₃), 30.19 (CH₃), 26.30 (CH₃), 20.21 (COCH₃). MS-DCI m/z : 382 (M+H)⁺. Anal. Calcd for C₂₂H₂₃NO₅: C: 69.28, H: 6.08, N: 3.67. Found: C: 69.19, H: 6.02, N: 3.54.

1-Benzoyloxy-1,2-dihydroisoacronycine (18). To a solution of **14** (30 mg, 0.09 mmol) in dry pyridine (1.5 mL) was added benzoic anhydride (90 mg, 0.40 mmol). The reaction mixture was stirred for 12 h at 60 °C and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue mixture was extracted with EtOAc-NaHCO₃ (sat.) and the organic layer was collected. The solvent was removed under reduced pressure and the remaining residue was purified by flash chromatography on silica gel with CH₂Cl₂ to give compound (**18**) (20 mg, 51%). mp 134 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1712, 1633, 1603, 1556, 1322, 1270 cm⁻¹. ¹H-NMR (CDCl₃, 400

MHz) δ : 8.49 (1H, d, J = 8 Hz, H-10), 8.06 (2H, d, J = 8 Hz, H-2',6'), 7.68 (1H, t, J = 8 Hz, H-8), 7.56 (1H, t, J = 8 Hz, H-4'), 7.44 (2H, d, J = 8 Hz, H-3',5'), 7.42 (1H, d, J = 8 Hz, H-7), 7.26 (1H, t, J = 8 Hz, H-9), 6.74 (1H, s, H-5), 6.60 (1H, dd, J = 4.5, 1.5 Hz, H-1), 3.93 (3H, s, OCH₃), 3.82 (3H, s, NCH₃), 2.37 (1H, dd, J = 15, 1.5 Hz, H-2a), 2.26 (1H, dd, J = 15, 4.5 Hz, H-2b), 1.57 (3H, s, CH₃), 1.53 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.88 (C-11), 165.68 (C₆H₅COO), 163.38 (C-12), 159.10 (C-4a), 146.92 (C-5a), 142.15 (C-6a), 133.70 (C-8), 132.84 (C-4'), 130.89 (C-1'), 129.96 (C-2',6'), 128.00 (C-3',5'), 127.75 (C-10), 124.02 (C-10a), 121.17 (C-9), 114.00 (C-7), 111.04 (C-11a), 108.08 (C-12a), 97.71 (C-5), 74.83 (C-3), 61.99 (OCH₃), 61.66 (C-1), 39.30 (C-2), 34.75 (NCH₃), 30.28 (CH₃), 26.28 (CH₃). MS-DCI m/z : 444 (M+H)⁺. Anal. Calcd for C₂₇H₂₅NO₅: C: 73.12, H: 5.68, N: 3.16. Found: C: 73.09, H: 5.59, N: 3.14.

1-Octadecanoyloxy-1,2-dihydroisoacronycine (17). To a solution of **14** (30 mg, 0.09 mmol) in dry pyridine (1.5 mL) was added octadecanoyl chloride (0.40 mL, 1.18 mmol). The reaction mixture was stirred for 3 h at rt and then the reagents were removed under reduced pressure (using a high vacuum pump). The remaining residue was purified by flash chromatography on silica gel with CH₂Cl₂ to give compound **17** (30 mg, 56%). mp 114 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1724, 1634, 1603, 1556, 1322, 1138 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.50 (1H, d, J = 8 Hz, H-10), 7.67 (1H, t, J = 8 Hz, H-8), 7.42 (1H, d, J = 8 Hz, H-7), 7.27 (1H, t, J = 8 Hz, H-9), 6.68 (1H, s, H-5), 6.31 (1H, dd, J = 5, 3 Hz, H-1), 3.98 (3H, s, OCH₃), 3.79 (3H, s, NCH₃), 2.37 (2H, m, H-2'), 2.22 (1H, dd, J = 15, 3 Hz, H-2a), 2.15 (1H, dd, J = 15, 5 Hz, H-2b), 1.66 (2H, m, H-3'), 1.50 (3H, s, CH₃), 1.48 (3H, s, CH₃), 1.44 - 1.26 (28H, H4'-H17'), 0.90 (3H, t, J = 7 Hz, H-18'). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.38 (C-11), 173.03 (C-1'), 162.89 (C-12), 159.06 (C-4a), 146.47 (C-5a), 142.03 (C-6a), 133.18 (C-8), 127.70 (C-10), 123.86 (C-10a), 121.22 (C-9), 114.14 (C-7), 111.30 (C-11a), 108.29 (C-12a), 97.57 (C-5), 75.42 (C-3), 62.37 (OCH₃), 61.43 (C-1), 38.92 (C-2), 34.74 (NCH₃), 34.53 (C-2'), 31.93, 29.67, 29.48, 29.36, 29.29, 26.91, 25.86 (C-3'-16'), 24.85 (2xCH₃), 22.69 (C-17'), 14.13 (C-18'). MS-DCI m/z : 606 (M+H)⁺. Anal. Calcd for C₃₈H₅₅NO₄: C: 75.33, H: 9.15, N: 2.31. Found: C: 75.29, H: 9.09, N: 2.27.

1-(2-Chloroethylcarbamoyloxy)-1,2-dihydroisoacronycine (19). To a solution of **14** (30 mg, 0.09 mmol) in dry pyridine (1.5 mL) was added 2-chloroethyl isocyanate (0.30 mL, 3.52 mmol). The reaction mixture was stirred for 48 h at rt and then the reagents were removed under reduced pressure (using a high vacuum pump). The remaining residue was purified by flash chromatography on silica gel with CH₂Cl₂ to give compound (**19**) (25 mg, 64%). mp 155 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 3320, 1696, 1634, 1603, 1556, 1328, 1136 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 8.50 (1H, d, J = 8 Hz, H-10), 7.68 (1H, t, J = 8 Hz, H-8), 7.43 (1H, d, J = 8 Hz, H-7), 7.27 (1H, t, J = 8 Hz, H-9), 6.67 (1H, s, H-5), 6.16 (1H, dd, J = 5, 3 Hz, H-1), 5.12 (1H, br s, N-H), 3.96 (3H, s, OCH₃), 3.76 (3H, s, NCH₃), 3.60 (2H, t, J = 5 Hz, H-2'), 3.54 (2H, t, J = 5 Hz, H-3'), 2.28 (1H, dd, J = 15, 3 Hz, H-2a), 2.12 (1H, dd, J = 15, 5 Hz,

H-2b), 1.49 (3H, s, CH₃), 1.47 (3H, s, CH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ: 176.42 (C-11), 162.75 (C-1'), 159.03 (C-12), 155.69 (C-4a), 146.35 (C-5a), 141.94 (C-6a), 133.23 (C-8), 127.54 (C-10), 123.64 (C-10a), 121.21 (C-9), 114.19 (C-7), 111.03 (C-11a), 108.35 (C-12a), 97.58 (C-5), 75.46 (C-3), 62.56 (C-1, OCH₃), 44.66 (C-2'), 42.71 (C-3'), 39.00 (C-2), 34.51 (NCH₃), 29.59 (CH₃), 25.77 (CH₃). MS-DCI *m/z*: 445 (M+H)⁺. Anal. Calcd for C₂₃H₂₅ClN₂O₅: C: 62.09, H: 5.66, N: 6.30, Cl: 7.97. Found: C: 62.19, H: 5.72, N: 6.34, Cl: 7.90.

1-(*n*-Dodecylcarbamoyloxy)-1,2-dihydroisoacronycine (20). To a solution of **14** (30 mg, 0.09 mmol) in dry pyridine (1.5 mL) was added *n*-dodecyl isocyanate (0.15 mL, 0.62 mmol). The reaction mixture was stirred for 48 h at 60 °C and then the reagents were removed under reduced pressure (using a high vacuum pump). The remaining residue was purified by flash chromatography on silica gel with cyclohexane: EtOAc (95: 5 to 80: 20) to give compound (**20**) (20 mg, 42%). mp 126 °C (cyclohexane-EtOAc). IR (CHCl₃) *v*_{max} 3330, 1690, 1634, 1604, 1556, 1328, 1137 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ: 8.45 (1H, dd, *J* = 8, 1.5 Hz, H-10), 7.65 (1H, td, *J* = 8, 1.5 Hz, H-8), 7.41 (1H, d, *J* = 8 Hz, H-7), 7.24 (1H, t, *J* = 8 Hz, H-9), 6.65 (1H, s, H-5), 6.14 (1H, dd, *J* = 5, 3 Hz, H-1), 4.63 (1H, br s, N-H), 4.00 (3H, s, OCH₃), 3.79 (3H, s, NCH₃), 3.17 (2H, t, *J* = 6 Hz, H-2'), 2.33 (1H, dd, *J* = 15, 3 Hz, H-2a), 2.15 (1H, dd, *J* = 15, 5 Hz, H-2b), 1.70-1.20 (20H, H3'-H12'), 1.51 (6H, s, 2xCH₃), 0.90 (3H, t, *J* = 6 Hz, H-13'). ¹³C-NMR (CDCl₃, 50 MHz) δ: 176.54 (C-11), 163.35 (C-12), 159.03 (C-4a), 155.95 (C-1'), 146.86 (C-5a), 142.02 (C-6a), 133.12 (C-8), 127.68 (C-10), 123.82 (C-10a), 121.18 (C-9), 114.12 (C-7), 111.73 (C-11a), 108.72 (C-12a), 97.47 (C-5), 75.53 (C-3), 62.48 (C-1), 62.11 (OCH₃), 41.05 (C-2'), 39.14 (C-2), 34.51 (NCH₃), 31.86, 30.17, 30.03, 29.59, 29.33 (C-3'-11'), 26.76 (2xCH₃), 22.68 (C-12'), 14.11 (C-13'). MS-DCI *m/z*: 551 (M+H)⁺. Anal. Calcd for C₃₃H₄₆N₂O₅: C: 71.97, H: 8.42, N: 5.09. Found: C: 71.99, H: 8.36, N: 5.00.

3-Oxo-12-demethylnoracronycine (6). To a solution of 1,3-dihydroxyacridone (**5**) (260 mg, 1.14 mmol) in H₂SO₄ (95%, 5 mL) was added malic acid (390 mg, 2.91 mmol). The reaction mixture was stirred for 20 min at 120 °C and then it was poured into ice. The precipitate was filtered and dissolved in NaOH (1N). This solution was extracted with CH₂Cl₂/H₂O and the organic layer was collected and evaporated. The solid residue was crystallized with EtOAc to give compound (**6**) (100 mg, 32%). mp 340 °C (decomp). IR (KBr) *v*_{max} 3520, 1733, 1635, 1603, 1556, 1328, 1130 cm⁻¹. ¹H-NMR (DMSO, 400 MHz) δ: 15.69 (1H, s, OH-6), 8.72 (1H, d, *J* = 9 Hz, H-1), 8.18 (1H, d, *J* = 8 Hz, H-8), 7.80 (2H, br s, H-10,11), 7.35 (1H, br s, H-9), 6.35 (1H, s, H-5), 6.28 (1H, d, *J* = 9 Hz, H-2). ¹³C-NMR (DMSO, 50 MHz) δ: 179.58 (C-7), 167.09 (C-3), 160.28 (C-6,4a), 143.26 (C-11a), 141.50 (C-12a), 139.92 (C-1), 133.56 (C-10), 124.58 (C-8), 122.26 (C-9), 120.72 (C-11), 119.69 (C-7a), 108.27 (C-2), 105.71 (C-6a), 100.43 (C-12b), 94.11 (C-5). MS-DCI *m/z*: 280 (M+H)⁺. Anal. Calcd for C₁₆H₉NO₄: C: 68.82, H: 3.25, N: 5.02. Found: C: 68.88, H: 3.42, N: 5.20.

Methylation of 6. To a solution of **6** (100 mg, 0.36 mmol) in dry acetone (12 mL) was added dimethyl sulfate (1.14 mL, 12 mmol) and 2.0 g (14.5 mmol) of anhydrous K₂CO₃. The reaction mixture was stirred for 24 h at rt and then it was filtered. The filtrate was extracted with CH₂Cl₂/H₂O and the organic layer was collected and evaporated. The remaining residue was purified by flash chromatography on silica gel with cyclohexane: EtOAc (75: 25 to 50: 50) to give compound (**7**) (54 mg, 49%), compound (**9**) (22 mg, 17%) and compound (**8**) (12 mg, 11%).

3-Oxoacronycine (7). mp 263 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1734, 1636, 1605, 1573, 1487, 1134 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz) δ : 8.37 (1H, dd, J = 8, 1.5 Hz, H-8), 8.03 (1H, d, J = 10 Hz, H-1), 7.70 (1H, td, J = 8, 1.5 Hz, H-10), 7.42 (1H, d, J = 8 Hz, H-11), 7.34 (1H, t, J = 8 Hz, H-9), 6.65 (1H, s, H-5), 6.24 (1H, d, J = 10 Hz, H-2), 4.03 (3H, s, OCH₃), 3.95 (3H, s, NCH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 176.89 (C-7), 164.32 (C-3), 160.13 (C-4a), 159.69 (C-6), 146.97 (C-12a), 144.27 (C-11a), 141.31 (C-1), 133.38 (C-10), 127.20 (C-8), 125.72 (C-7a), 123.16 (C-9), 116.40 (C-11), 112.72 (C-6a), 109.70 (C-2), 102.29 (C-12b), 94.41 (C-5), 56.86 (OCH₃), 45.60 (NCH₃). MS-EI m/z : 307 (M⁺)⁺. Anal. Calcd for C₁₈H₁₃NO₄: C: 70.35, H: 4.26, N: 4.56. Found: C: 70.46, H: 4.35, N: 4.69.

4,6-Dimethoxy-3,2,1-deacridine-1,7-dione (9). mp 231 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1725, 1645, 1602, 1562, 1465 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz) δ : 9.10 (1H, d, J = 10 Hz, H-3), 8.33 (1H, ddd, J = 9, 2, 1 Hz, H-8), 8.17 (1H, ddd, J = 9, 1.5, 1 Hz, H-11), 7.82 (1H, ddd, J = 9, 7, 2 Hz, H-10), 7.56 (1H, ddd, J = 9, 7, 1.5 Hz, H-9), 6.74 (1H, s, H-5), 6.40 (1H, d, J = 10 Hz, H-2), 4.13 (3H, s, 4-OCH₃), 4.11 (3H, s, 6-OCH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 177.89 (C-7), 163.44 (C-6), 161.75 (C-1), 159.76 (C-4), 158.22 (C-6a), 150.42 (C-11a), 147.87 (C-12a), 141.54 (C-3), 131.62 (C-10), 129.11 (C-11), 125.68 (C-9), 122.94 (C-8), 121.61 (C-7a), 111.55 (C-2), 108.90 (C-3a), 95.63 (C-5), 64.28 (4-OCH₃), 56.71 (6-OCH₃). MS-EI m/z : 307 (M⁺)⁺. Anal. Calcd for C₁₈H₁₃NO₄: C: 70.35, H: 4.26, N: 4.56. Found: C: 70.51, H: 4.42, N: 4.59.

Methyl 1,3-dimethoxy-10-methyl-9-oxo-10H-acridinyl-4-propenoate (8). mp 195 °C (cyclohexane-EtOAc). IR (CHCl₃) $\nu_{\max}$ 1699, 1617, 1603, 1575, 1466, 1315 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz) δ : 8.33 (1H, dd, J = 8, 1.5 Hz, H-8), 7.59 (1H, td, J = 8, 1.5 Hz, H-6), 7.25 (1H, d, J = 8 Hz, H-5), 7.22 (1H, t, J = 8 Hz, H-7), 7.00 (1H, d, J = 12 Hz, H-1'), 6.32 (1H, s, H-2), 6.09 (1H, d, J = 12 Hz, H-2'), 4.03 (3H, s, 1-OCH₃), 3.91 (3H, s, 3-OCH₃), 3.61 (3H, s, NCH₃), 3.31 (1H, s, 3'-OCH₃). ¹³C-NMR (CDCl₃, 50 MHz) δ : 177.63 (C-9), 166.96 (C-3'), 163.08 (C-1), 161.13 (C-3), 149.10 (C-4a), 144.63 (C-10a), 136.90 (C-1'), 132.80 (C-6), 126.98 (C-8), 125.29 (C-8a), 121.79 (C-7), 120.44 (C-2'), 116.05 (C-5), 110.59 (C-9a), 105.21 (C-4), 88.91 (C-2), 56.27 (1-OCH₃), 55.76 (3-OCH₃), 51.20 (3'-OCH₃), 45.25 (NCH₃). MS-EI m/z : 353 (M⁺)⁺. Anal. Calcd for C₂₀H₁₉NO₅: C: 67.98, H: 5.42, N: 3.96. Found: C: 67.99, H: 5.32, N: 3.88.

Cell Culture and Cytotoxicity. L1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and 10 mM HEPES buffer (pH =7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.¹² Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC₅₀, the concentration needed to reduce by 50% the optical density of treated cells with respect to the optical density of untreated controls.

REFERENCES AND NOTES

1. G. K Hughes, F. N. Lahey, J. R. Price, and L. J. Webb, *Nature*, 1948, **62**, 223.
2. P. L. Macdonald and A. V. Robertson, *Aust. J. Chem.*, 1966, **19**, 275.
3. G. H. Svoboda, *Lloydia*, 1966, **29**, 206.
4. G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *J. Pharm. Sci.*, 1966, **55**, 758.
5. A. L. Skaltsounis, S. Mitaku, and F. Tillequin, *Acridone Alkaloids: The Alkaloids*, Vol. 54, ed. by G. Cordell, Academic Press, 2000.
6. F. Tillequin, S. Michel, and A. L. Skaltsounis, "Alkaloids: Chemical and biological properties" Vol. 12, ed. by S. W. Pelletier, Elsevier, New York, 1998, pp. 1-102.
7. C. S Oh and C. V. Greco, *J. Heterocycl. Chem.*, 1970, 261.
8. J. Reisch, H. M. T. B. Herath, and N. S. Kumar, *Liebigs Ann.Chem.*, 1991, 685.
9. E. Melliou, P. Magiatis, S. Mitaku, A. L. Skaltsounis, A. Pierré, G. Atassi, and P. Renard, *Bioorg. Med. Chem.*, 2001, **9**, 607.
10. *N*-Methyl-1,3-dihydroxyacridone was prepared as described in: J. Hlubucek, E. Ritchie, and W. C. Taylor, *Aust. J. Chem.*, 1970, **23**, 1881.
11. S. Mitaku, A. L. Skaltsounis, F. Tillequin, and M. Koch, *Planta Med.*, 1988, **54**, 24.
12. A. Pierré, T. A. Dunn, L. Kraus-Berthier, S. Léonce, D. Saint-Dizier, G. Regnier, A. Dhainaut, M. Berlion, J. P. Bizzari, and G. Atassi, *Invest. New Drug*, 1992, **10**, 137.