Synthesis and Cytotoxic Activity of Acronycine Derivatives Modified at the Pyran Ring

Abdelhakim ELOMRI,[a] Alexios-Leandros SKALTSOUNIS,[a,1)] Sylvie MICHEL,[a] François TILLEQUIN,*[,a] Michel KOCH,[a] Yves ROLLAND,[b] Alain PIERRÉ,[b] and Ghanem ATASSI[b]

Laboratoire de Pharmacognosie de l'Université René Descartes,[a] U.R.A. au C.N.R.S. n°1310, Faculté des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, F-75006 Paris, France and Institut de Recherche Servier,[b] 11 rue des Moulineaux, F-92150 Suresnes, France. Received April 15, 1996; accepted June 18, 1996

Nitration of acronycine (1) and 6-demethoxyacronycine (3) afforded 2-nitroacronycine (2) and 2-nitro-6-demethoxyacronycine (4), respectively. Reduction of 2-nitroacronycine yielded, depending on the conditions, 2-nitro-1,2-dihydroacronycine (5), 2-oxo-1,2-dihydroacronycine oxime (7) or 2-amino-1,2-dihydroacronycine (6). This latter was readily converted into 2-dimethylamino-1,2-dihydroacronycine (8), 2-acetylamino-1,2-dihydro-acronycine (9) and 2-benzoylamino-1,2-dihydroacronycine (10).

The cytotoxicity of these compounds was evaluated against L1210 leukemia cells. Compounds 2 and 7 were 300- and 10-fold more potent than acronycine in inhibiting L1210 cell proliferation, respectively. Compound 2 was devoid of antitumor activity against P388 leukemia and C38 colon adenocarcinoma.

Key words acronycine; nitration; 2-amino-1,2-dihydroacronycine; cytotoxicity

The acridone alkaloid acronycine (**1**), which was first isolated from *Acronychia baueri* SCHOTT (Rutaceae) in 1948 was later found to be a potent anticancer agent.[2-5)] It is of interest because of its broad spectrum of activity, including numerous solid tumors.[3-6)] Nevertheless, clinical trials have been severely hampered by its very low water-solubility and have therefore given only poor results.[7)]

Despite the interest in acronycine, little chemical investigation has been conducted in order to modify it at the pyran ring. Previously described compounds modified at the 1 and/or 2 positions in that series include only dihydro,[8)] hydroxydihydro,[9)] dihydroxydihydro,[9)] bromo,[8)] nitro and dihydronitro derivatives.[3,10)]

The nitro and dihydronitro derivatives appear of particular potential interest, since soluble amino and amido compounds should be obtainable from them.

Nitration of acronycine using nitric acid leads to a mononitroacronycine whose structure has been reported as 1-nitroacronycine by Drummond and Lahey[11)] and as 2-nitroacronycine by Svoboda.[3)] This compound was described as biologically inactive by Svoboda.[3)] It has been more recently evaluated by Cordell *et al.*,[11)] who found that nitroacronycine demonstrated a greater activity than acronycine itself against a battery of cultured mammalian tumor cells.

This paper deals with the synthesis and cytotoxic properties of amino and amido derivatives in the acronycine series. These compounds have been synthesized from mononitroacronycine, whose chemical structure and cytotoxic properties have been reinvestigated.

Chemistry

Treatment of acronycine (**1**) with fuming nitric acid in acetic acid at 0 °C led to mononitroacronycine in 90% yield.[3,10)] Observation of a strong cross peak between the singlet of the pyran proton and the signal of the N-CH_3 group in two dimensional nuclear Overhauser effect spectroscopy (2D NOESY) ^{1}H-NMR[12)] permitted us to establish unambiguously the structure of this compound as 2-nitroacronycine (**2**). In a similar way, nitration of 6-demethoxyacronycine (**3**)[13)] afforded 2-nitro-6-demethoxyacronycine (**4**).

The reduction of 2-nitroacronycine (**2**) was performed

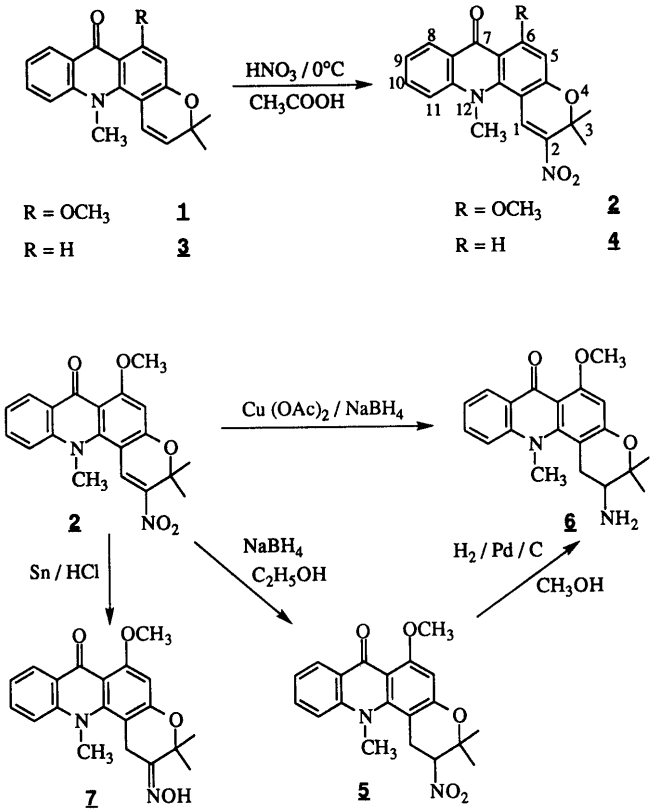

Chart 1

* To whom correspondence should be addressed.

Table 1. Inhibition of L1210 Cell Proliferation by Compounds **2** and **4**—**10** in Comparison with Acronycine (**1**) and 6-Demethoxyacronycine (**3**)

Compound	1	2	3	4	5	6	7	8	9	10
IC_{50} (μM)	27	0.09	29.9	13.7	32.7	12.5	2.8	>50	>50	47.86

Table 2. Antitumor Activities of Compound **2** in Comparison with Acronycine (**1**) against P388 Leukemia and C38 Colon Carcinoma in Mice

Compound	1	2
P388 T/C %[a]	125 (200 mg/kg)[c]	113 (50 mg/kg)[c]
C38 T/C %[b]	4 (200 mg/kg)[c]	137 (25 mg/kg)[c]

a) Survival. *b*) Tumor volume. *c*) Maximum tolerated dose.

in two steps. A first reduction of **2** with sodium borohydride yielded the known 2-nitro-1,2-dihydroacronycine (**5**)[3] which, in turn, could be reduced to 2-amino-1,2-dihydroacronycine (**6**) by hydrogenation at room temperature using Pd/C as a catalyst. Alternatively, **6** could be prepared more easily from **2** by direct reduction using sodium borohydride in the presence of cupric acetate.[14] When this latter reaction was carried out for a short time (<2 h), small amounts (yield <5%) of the intermediate 2-oxo-1,2-dihydroacronycine oxime (**7**) could be isolated from the reaction mixture in addition to **6**. In contrast, **7** was prepared in high yield by reduction of 2-nitroacronycine (**2**) with tin and hydrochloric acid in methanol.

Treatment of 2-amino-1,2-dihydroacronycine (**6**) with formaldehyde and sodium cyanoborohydride[15] smoothly afforded 2-dimethylamino-1,2-dihydroacronycine (**8**).

Finally, both aliphatic and aromatic amides were obtained in excellent yields from 2-amino-1,2-dihydroacronycine (**6**) upon treatment with acid anhydrides in pyridine. This reaction is exemplified by the preparation of 2-acetylamino-1,2-dihydroacronycine (**9**) and 2-benzoylamino-1,2-dihydroacronycine (**10**) using acetic anhydride and benzoic anhydride, respectively.

Pharmacology Compounds **2** and **7** were strongly cytotoxic: they were 300- and 10-fold more potent than acronycine in inhibiting the proliferation of L1210 cells, respectively. Compounds **8**, **9**, and **10** were devoid of antiproliferative activity, and the other compounds (**3**, **4**, **5**, **6**) were about as potent as acronycine (Table 1). Compound **2** was devoid of antitumor activity against P388 leukemia and C38 colon adenocarcinoma in mice (Table 2), unlike **1**, which is moderately active against P388 leukemia and markedly active against C38 colon cells.

Results and Discussion

Considering the structure–cytotoxic activity relationships, it appears that only compounds bearing both a methoxy substituent at the 6-position and a 1,2-double bond such, as **2** and **7** (as its tautomeric 2-hydroxylaminoacronycine form)[16] exhibit a significantly more potent activity than acronycine itself. The most cytotoxic compound, 2-nitroacronycine (**2**) would be therefore worth testing in *in vivo* experimental models, since discrepancies exist in the literature results published.[3,11] The strong *in vitro* cytotoxicity of **2** and its lack of antitumor activity *in vivo* are in good agreement with previous studies.[3,4]

Experimental

Chemistry Mass spectra (MS) were recorded with a Nermag R-10-10C spectrometer using electron impact (EI)- and/or chemical ionization (CI)-MS (reagent gas: NH_3) techniques. UV spectra (λ_{max} in nm) were determined in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained in potassium bromide pellets on a Perkin-Elmer 257 instrument. ^{1}H-NMR (δ [ppm], J [Hz]) and ^{13}C-NMR spectra were recorded at 300 and 75 MHz respectively, using a Bruker AC-300 spectrometer. Column chromatography was conducted using flash Silica gel 60 Merck (40—63 μm) with an overpressure of 300 mbar.

2-Nitroacronycine (2) In a typical experiment, cold fuming nitric acid (1.14 ml) was added dropwise at 0 °C to a solution of acronycine (**1**) (0.350 g, 0.11 mmol) in glacial acetic acid (11.5 ml). The reaction mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature. It was diluted with MeOH (20 ml) and concentrated *in vacuo* to *ca.* 15 ml at low temperature (<25 °C). The solution was neutralized by addition of 34% aqueous NH_4OH and extracted with CH_2Cl_2 (2 × 15 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Recrystallization from MeOH afforded **2** as yellow needles (0.362 g, 90%). mp: 226 °C; (lit.[10]: 222 °C; lit.[3]: 232—235 °C). UV and IR data were identical with those previously published.[3] ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.85 (s, 6H, $(CH_3)_2$), 3.90 (s, 3H, N–CH_3), 4.00 (s, 3H, CH_3O), 6.30 (s, 1H, C5-H), 7.32 (td, 1H, J=8 Hz, J'=2 Hz, C9-H), 7.40 (dd, 1H, J=8 Hz, J'=2 Hz, C11-H), 7.69 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.04 (s, 1H, C1-H), 8.36 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.3 (q, $(CH_3)_2$), 47.2 (q, N–CH_3), 56.7 (q, CH_3O), 78.7 (s, C-3), 93.8 (d, C-5), 100.2 (s, C-12b), 111.02 (s, C-6a), 116.3 (d, C-11), 123.1 (d, C-9), 125.7 (s, C-7a), 127.1 (d, C-8), 128.2 (d, C-1), 133.2 (d, C-10), 139.5 (s, C-2), 143.8 (s, C-11a), 148.8 (s, C-12a), 160.6 (s, C-4a), 166.9 (s, C-6), 176.5 (s, C-7).

2-Nitro-6-demethoxyacronycine (4) Nitration of 6-demethoxyacronycine (**3**) (0.15 g, 0.44 mmol) under conditions similar to those described for the preparation of **2** afforded **4** (0.072 g, 49%) as an amorphous yellow solid. IR (KBr) cm^{-1}: 2930, 1600, 1495, 1260. UV λ_{max}^{MeOH} nm (log ε): 263 (4.34), 307 (3.90), 421 (3.45). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.08 (s, 6H, $(CH_3)_2$), 4.13 (s, 3H, N–CH_3), 6.87 (d, 1H, J=8 Hz, C5-H), 7.37 (ddd, 1H, J=8 Hz, J'=7 Hz, J''=1 Hz, C9-H), 7.48 (d, 1H, J=8 Hz, J'=1 Hz, C11-H), 7.75 (ddd, 1H, J= 8Hz, J'=7 Hz, J''=2 Hz, C10-H), 8.06 (s, 1H, C1-H), 8.41 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H), 8.44 (d, 1H, J=8 Hz, C6-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.2 (q, $(CH_3)_2$), 44.0 (q, N–CH_3), 78.0 (s, C-3), 106.4 (s, C-12b), 112.1 (d, C-5), 116.7 (d, C-11), 119.2 (s, C-6a), 123.5 (s, C-7a), 126.7 (d, C-6), 127.2 (d, C-8), 127.7 (d, C-1), 127.9 (d, C-9), 133.9 (d, C-10), 141.9 (s, C-2), 145.0 (s, C-11a), 145.7 (s, C-12a), 160.1 (s, C-4a), 176.9 (s, C-7). MS m/z: 336 ($M^+\cdot$). *Anal.* Calcd for $C_{19}H_{16}N_2O_4$: C, 67.74; H, 4.74; N, 8.28. Found: C, 67.84; H, 4.79; N, 8.32.

2-Nitro-1,2-dihydroacronycine (5) Treatment of **2** with sodium borohydride under the conditions previously described[3] afforded **5** as needles, mp: 216—219 °C (lit.[3]: 222—224 °C); UV and IR data were identical with those previously published.[3] ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.55 (s, 3H, $(CH_3)_a$), 1.60 (s, 3H, $(CH_3)_b$), 3.25 (dd, 1H, J=16 Hz, J'=5 Hz, C1-H_a), 3.63 (dd, 1H, J=16 Hz, J'=10 Hz, C1-H_b), 3.80 (s, 3H, N–CH_3), 3.95 (s, 3H, CH_3O), 4.72 (dd, 1H, J=10Hz, J'=5 Hz, C2-H), 6.29 (s, 1H, C5-H), 7.24 (td, 1H, J=8 Hz, J'=2 Hz, C9-H), 7.34 (dd, 1H, J=8 Hz, J'=2 Hz, C11-H), 7.62 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.31 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 20.7 (q, $(CH_3)_a$), 26.1 (q, $(CH_3)_b$), 27.5 (t, C-1), 44.3 (q, N–CH_3), 56.2 (q, CH_3O), 74.9 (s, C-3), 86.1 (d, C-2), 94.9 (d, C-5), 97.5 (s, C-12b), 111.7 (s, C-6a), 116.4 (d, C-11), 122.1 (d, C-9), 125.9 (s, C-7a), 126.8 (d, C-8), 132.7 (d, C-10), 145.7 (s, C-11a), 149.8 (s, C-12a), 157.2 (s, C-4a), 161.3 (s, C-6), 177.7 (s, C-7).

2-Amino-1,2-dihydroacronycine (6) Method a: A saturated aqueous solution of $Cu(OAc)_2$ (containing some solid) (1 ml) was added to a

solution of **2** (0.080 g, 0.22 mmol) in MeOH (5 ml). Small portions of $NaBH_4$ (0.090 g) were added with stirring at room temperature over 2 h, then EtOAc (10 ml) was added. The reaction mixture was washed with aqueous $NaHCO_3$. The aqueous layer was further extracted with EtOAc (10 ml) and the combined organic fractions were dried over anhydrous Na_2SO_4, filtered and evaporated. Column chromatography of the residue on silica gel (CH_2Cl_2–MeOH, 99 : 1) afforded **6** (45 mg, 60%) as an amorphous solid. UV λ_{max}^{MeOH} nm (log ε): 272 (4.46), 298 (3.97), 318 (3.86), 382 (3.69). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.41 (s, 3H, $(CH_3)_a$), 1.49 (s, 3H, $(CH_3)_b$), 2.57 (s, 2H, NH_2), 2.75 (dd, 1H, J=14 Hz, J'=6 Hz, C1-H_a), 2.95 (dd, 1H, J=6 Hz, J'=5 Hz, C2-H), 3.01 (dd, 1H, J=14 Hz, J'=5 Hz, C1-H_b), 3.75 (s, 3H, N–CH_3), 3.92 (s, 3H, CH_3O), 6.24 (s, 1H, C5-H), 7.22 (td, 1H, J=8 Hz, J'=2 Hz, C9-H), 7.32 (dd, 1H, J=8 Hz, J'=2 Hz, C11-H), 7.59 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.30 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 26.1 (q, $(CH_3)_2$), 29.6 (t, C-1), 44.1 (q, N–CH_3), 51.8 (d, C-2), 56.1 (q, CH_3O), 77.9 (s, C-3), 95.1 (d, C-5), 100.0 (s, C-12b), 111.1 (s, C-6a), 116.2 (d, C-11), 121.6 (d, C-9), 125.8 (s, C-7a), 126.8 (d, C-8), 132.4 (d, C-10), 147.8 (s, C-11a), 150.2 (s, C-12a), 158.5 (s, C-4a), 160.7 (s, C-6), 177.8 (s, C-7). MS *m/z*: 338 (M^+·). *Anal.* Calcd for $C_{20}H_{22}N_2O_3$: C, 70.98; H, 6.55; N, 8.27. Found : C, 70.88; H, 6.52; N, 8.17.

Method b: A solution of **5** (0.033 g, 0.08 mmol) in MeOH (3 ml) was added to a suspension of Pd/C (5% Pd, 30 mg) in MeOH–CH_3COOH (3 : 1). The mixture was stirred for 3 h under H_2 (1 atm), then filtered over Celite and the filtrate was evaporated. The residue was taken up in CH_2Cl_2 (15 ml) and 10% NH_4OH (10 ml). The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure. Column chromatography of the residue on silica gel (eluent: CH_2Cl_2–MeOH, 99 : 3) afforded **6** (0.017 g, 54%).

2-Oxo-1,2-dihydroacronycine Oxime (7) Tin powder (0.2 g, 1.6 mmol) was added in portions with stirring to a solution of **2** (0.060 g, 0.16 mmol) in MeOH (6 ml) and aqueous 2 N HCl (2 ml). After addition was complete (*ca.* 30 min), the mixture was stirred for 1 h. The precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was alkalinized with 10% NH_4OH and extracted with CH_2Cl_2 (3 × 10 ml). The combined CH_2Cl_2 fractions were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. Recrystallization of the residue from MeOH–CH_2Cl_2 gave **7** as needles (0.47 g, 79%); mp 234 °C. IR (KBr) cm^{-1}: 3460, 3000, 2960, 1730, 1600, 1220. UV λ_{max}^{MeOH} nm (log ε): 263 (4.37), 382 (3.66). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.48 (s, 6H, $(CH_3)_2$), 3.73 (s, 3H, N–CH_3), 3.77 (s, 2H, C1-H), 3.97 (s, 3H, CH_3O), 6.46 (s, 1H, C5-H), 7.23 (td, 1H, J=8 Hz, J'=1 Hz, C9-H), 7.33 (dd, 1H, J=8 Hz, J'=1 Hz, C11-H), 7.50 (s, br D_2O exch., 1H, N–OH), 7.62 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.30 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 22.9 (q, $(CH_3)_a$), 23.2 (q, $(CH_3)_b$), 24.3 (t, C-1), 45.0 (q, N–CH_3), 56.1 (q, CH_3O), 78.6 (s, C-3), 95.9 (d, C-5), 100.2 (s, C-12b), 111.4 (s, C-6a), 116.5 (d, C-11), 122.5 (d, C-9), 125.3 (s, C-7a), 127.0 (d, C-8), 133.3 (d, C-10), 144.9 (s, C-11a), 148.8 (s, C-12a), 159.4 (s, C-2), 160.1 (s, C-4a), 162.4 (s, C-6), 177.2 (s, C-7). MS *m/z*: 352 (M^+·). *Anal.* Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.94. Found: C, 68.13; H, 5.67; N, 7.89.

2-Dimethylamino-1,2-dihydroacronycine (8) Sodium cyanoborohydride (0.6 g, 9.5 mmol) was added to a suspension of **6** hydrochloride (0.1 g, 0.27 mmol) in HCHO–AcOH (3 : 7) (4 ml). The reaction mixture was stirred at room temperature for 30 min, diluted with water (5 ml), alkalinized with aqueous NH_4OH (10 ml), and extracted with $CHCl_3$ (2 × 15 ml). The organic layers were collected, dried and evaporated, and the residue was chromatographed on a silica gel column (eluent: CH_2Cl_2–MeOH, 99 : 1). The eluates were pooled and evaporated to afford **8** (65 mg, 70%) as an amorphous solid. IR (KBr) cm^{-1}: 2960, 1610, 1340, 1220. UV λ_{max}^{MeOH} nm (log ε): 271 (4.43), 299 (3.78), 320 (3.70), 382 (3.64). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.48 (s, 3H, $(CH_3)_a$), 1.50 (s, 3H, $(CH_3)_b$), 2.30 (s, 6H, $(CH_3)_2N$), 2.66 (dd, 1H, J=8 Hz, J'=6 Hz, C2-H), 2.90 (dd, 2H, J=16 Hz, J'=8 Hz, C1-H_a; dd, J=16 Hz, J'=6 Hz, C1-H_b), 3.80 (s, 3H, N–CH_3), 3.91 (s, 3H, CH_3O), 6.22 (s, 1H, C5-H), 7.21 (td, 1H, J=8 Hz, J'=2 Hz, C9-H), 7.37 (dd, 1H, J=8 Hz, J'=2 Hz, C11-H), 7.60 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.32 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 21.6 (q, $(CH_3)_a$), 22.6 (q, $(CH_3)_b$), 27.2 (t, C-1), 43.1 (q, N-$(CH_3)_2$), 44.3 (q, N–CH_3), 56.1 (q, CH_3O), 63.4 (d, C-2), 78.9 (s, C-3), 95.2 (d, C-5), 101.2 (s, C-12b), 111.1 (s, C-6a), 116.4 (d, C-11), 121.7 (d, C-9), 125.9 (s, C-7a), 127.0 (d, C-8), 132.4 (d, C-10), 146.0 (s, C-11a), 150.0 (s, C-12a), 159.0 (s, C-4a), 160.8 (s, C-6), 177.9 (s, C-7). MS *m/z*: 366 (M^+·). *Anal.* Calcd for $C_{22}H_{26}N_2O_3$: C, 72.10; H, 7.15; N, 7.64. Found: C, 72.18; H, 7.18; N, 7.74.

2-Acetamido-1,2-dihydroacronycine (9) Ac_2O (1 ml) was added to a solution of **6** hydrochloride (40 mg, 0.11 mmol) in pyridine (1 ml). The reaction mixture was left overnight at room temperature. After usual work-up, the mixture afforded **9** (36 mg, 87%) as an amorphous powder. IR (KBr) cm^{-1}: 2910, 2940, 1630, 1600, 760. UV λ_{max}^{MeOH} nm (log ε): 271 (4.64), 297 (4.03), 318 (3.96), 379 (3.48). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.50 (s, 3H, $(CH_3)_a$), 1.55 (s, 3H, $(CH_3)_b$), 2.03 (s, 3H, CH_3CO), 2.71 (dd, 1H, J=16 Hz, J'=3 Hz, C1-H_a), 3.33 (dd, 1H, J=16 Hz, J'=6 Hz, C1-H_b), 3.47 (s, 3H, N–CH_3), 3.72 (s, 3H, CH_3O), 4.50 (ddd, 1H, J=10 Hz, J'=6 Hz, J'=3 Hz, C2-H), 5.87 (s, 1H, C5-H), 6.89 (d, 1H, J=10 Hz, H–N), 7.27 (td, 1H, J=8 Hz, J'=1 Hz, C9-H), 7.32 (dd, 1H, J=8 Hz, J'=1 Hz, C11-H), 7.62 (td, 1H, J=8 Hz, J'=2 Hz, C10-H), 8.30 (dd, 1H, J=8 Hz, J'=2 Hz, C8-H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 22.8 (q, $(CH_3)_a$), 25.0 (q, $(CH_3)_b$), 25.3 (t, C-1), 29.5 (q, CH_3-CO), 44.0 (q, N–CH_3), 47.4 (d, C-2), 52.8 (q, CH_3O), 77.4 (s, C-3), 95.0 (d, C-5), 98.6 (s, C-12b), 111.1 (s, C-6a), 116.2 (d, C-11), 121.9 (d, C-9), 125.6 (s, C-7a), 126.8 (d, C-8), 132.7 (d, C-10), 145.6 (s, C-11a), 150.5 (s, C-12a), 158.4 (s, C-4a), 160.0 (s, C-6), 171.1 (s, CO–NH), 177.2 (s, C-7). MS *m/z*: 380 (M^+·). *Anal.* Calcd for $C_{22}H_{24}N_2O_4$: C, 69.45; H, 6.36; N, 7.36. Found: C, 69.40; H, 6.27; N, 7.29.

2-Benzoylamido-1,2-dihydroacronycine (10) Benzoic anhydride (0.12 g, 0.55 mmol) was added to a solution of **6** hydrochloride (20 mg, 0.55 mmol) in pyridine (1 ml). The reaction mixture was stirred overnight at room temperature, and evaporated. Chromatography of the residue on a silica gel column (eluent: CH_2Cl_2), afforded **10** (21 mg, 89%) as an amorphous solid. UV λ_{max}^{MeOH} nm (log ε): 271 (4.42), 295 (3.90), 381 (3.63). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.51 (s, 3H, $(CH_3)_a$), 1.58 (s, 3H, $(CH_3)_b$), 2.88 (dd, 1H, J=16 Hz, J'=3 Hz, H_a-C1), 3.42 (dd, 1H, J=16 Hz, J'=5 Hz, H_b-C1), 3.77 (s, 3H, N–CH_3), 3.88 (s, 3H, CH_3O), 4.64 (ddd, 1H, J=9 Hz, J'=5 Hz, J''=3 Hz, H-C2), 6.26 (s, 1H, H-C5), 6.36 (d, 1H, J=9 Hz, H–N), 7.22 (td, 1H, J=9 Hz, J'=1 Hz, H-C9), 7.34 (t, 2H, J=9 Hz, H-C3′, H-C5′), 7.44 (tt, 1H, J=8 Hz, J'=2 Hz, H-C4′), 7.49 (dd, 1H, J=8 Hz, J'=1 Hz, H-C11), 7.66 (dd, 1H, dd, J=8 Hz, J'=2 Hz, H-C10), 8.13 (dd, 2H, J=8 Hz, J'=2 Hz, H-C2′, H-C6′), 8.31 (dd, 1H, J=8 Hz, J'=2 Hz, H-C8). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 24.7 (q, $(CH_3)_a$), 24.9 (t, C-1), 29.5 (q, $(CH_3)_b$), 44.2 (q, N–CH_3), 48.3 (d, C-2), 56.0 (q, CH_3O), 77.4 (s, C-3), 95.1 (d, C-5), 98.4 (s, C-12b), 111.6 (s, C-6a), 116.4 (d, C-11), 121.9 (d, C-9), 125.7 (s, C-7a), 126.9 (d, C-8), 128.4 (d, C-2′+C-6′), 128.5 (d, C-3′+C-5′), 130.1 (d, C-4′), 132.6 (s, C-1′), 133.5 (d, C-10), 145.7 (s, C-11a), 154.3 (s, C-12a), 158.1 (s, C-4a), 160.9 (s, C-6), 167.4 (s, CO-NH), 178.1 (s, C-7). CI-MS *m/z*: 443 ($M+H)^+$. *Anal.* Calcd for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33. Found: C, 73.38; H, 5.86; N, 6.24.

Biological Pharmacology Cytotoxicity: Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by microculture tetrazolium assay essentially as described.[17] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} (mean, n=3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

Antitumor Activity: The two tumors used were provided by the National Cancer Institute (Frederick, MD, U.S.A.). The test compounds were formulated as suspensions in 1% Tween 80 in water (v/v). For the P388 leukemia, 10^6 cells per B6D2F1 mouse (6 mice per group) were inoculated i.p. and the compounds were administered i.p. 24 h later, at doses ranging from 50 to 300 mg/kg (**1**) or 12.5 to 150 mg/kg (**2**). The results are given as % T/C survival, (100 × median survival times of treated/control animals) obtained at the maximum tolerated dose. For the C38 colon adenocarcinoma, tumor fragments of approximately 30 mg were implanted s.c. into B6D2F1 mice (9 mice per group). The compounds were administered i.p. on days 2 and 9, at the maximum tolerated doses. The tumors were measured on day 23 and the results are expressed as % T/C tumor volume (100 × median tumor volume of treated/control animals).

References

1) Present address: *Division de Pharmacognosie, Département de Pharmacie, Université d'Athènes, Panepistimiopolis, Zografou, Athènes 15771, Greece.*
2) Hughes G. K., Lahey F. N., Price J. R., Webb L. J., *Nature* (London), **162**, 223—224 (1948).

3) Svoboda G. H., *Lloydia*, **29**, 206—224 (1966).
4) Svoboda G. H., Poore G. A., Simpson P. J., Boder G. B., *J. Pharm. Sci.*, **55**, 758—768 (1966).
5) Suffness M., Cordell G. A., "The Alkaloids," Vol. XXV, ed. by Brossi A., Academic Press, New York, 1985, pp. 1—355.
6) Dorr R. T., Liddil J. D., Von Hoff D. D., Soble M., Osborne C. K., *Cancer Res.*, **49**, 340—344 (1989).
7) Scarffe J. H., Beaumont A. R., Gowther D., *Cancer. Treat. Rep.*, **67**, 93—94 (1983).
8) Brown R. D., Drummond L. J., Lahey F. N., Thomas W. C., *Aust. J. Sci. Res. A 2*, **1949**, 622—629.
9) Mitaku S., Skaltsounis A.-L., Tillequin F., Koch M., *Planta Med.*, **54**, 24—27 (1988).
10) Drummond L. J., Lahey F. N., *Aust. J. Sci. Res. A 2*, **1949**, 630—637.
11) Shieh H. L., Pezzuto G. M., Cordell G. A., *Chem.-Biol. Interactions*, **81**, 35—55 (1992).
12) Neuhaus D., Williamson M., "The Nuclear Overhauser Effect in Structural and Conformational Analysis," Verlag Chemie, New York, 1989.
13) Elomri A., Michel S., Tillequin F., Koch M., *Heterocycles*, **34**, 799—806 (1992).
14) Cowan J. A., *Tetrahedron Lett.*, **27**, 1205—1208 (1986).
15) Tillequin F., Koch M., *Phytochemistry*, **18**, 1559—1561 (1979).
16) Erlenmeyer H., Weber H. M., *Helv. Chim. Acta*, **21**, 614—615 (1938).
17) Pierré A., Kraus-Berthier L., Atassi Gh., Cros S., Poupon M. F., Lavielle G., Berlion M., Bizzari J. P., *Cancer Res.*, **51**, 2312—1318 (1991).