Synthesis and Cytotoxic Activity of 1-Alkoxy- and 1-Amino-2-hydroxy-1,2-dihydroacronycine Derivatives

Prokopios Magiatis,[a] Sofia Mitaku,[a] Alexios-Leandros Skaltsounis,*[,a] François Tillequin,[b] Michel Koch,[b] Alain Pierré,[c] and Ghanem Atassi[c]

Laboratory of Pharmacognosy, University of Athens,[a] Panepistimiopolis, Zografou, GR-15771 Athens, Greece, Laboratoire de Pharmacognosie de l'Université René Descartes,[b] URA au CNRS No 1310, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, F-75006 Paris, France, and Institut de Recherches SERVIER,[c] 11 rue des Moulineaux, 92150 Suresnes, France. Received November 2, 1998; accepted January 26, 1999

Sixteen new derivatives of the natural alkaloid acronycine, bearing 1-alkoxy or 1-amino and 2-hydroxy groups, were synthesized in order to clarify the role of the C-1 substitution. Studies on the cytotoxic activity of compounds 4—19 were carried out *in vitro* on L-1210 cells. Structure–activity relationships are discussed.

Key words acronycine; cytotoxicity; alkaloid; acridone

The acridone alkaloid acronycine (**1**), first isolated from *Acronychia baueri* Schott (Rutaceae) in 1948[1,2] exhibits a broad spectrum of activity against numerous solid tumors including sarcoma, myeloma, carcinoma and melanoma.[3—5] Nevertheless, clinical trials gave only poor results,[6] most probably due to the moderate potency of acronycine and its very low water-solubility which excludes parenteral formulation of the drug. Early structure–activity studies clearly indicated that the 1,2-double bond on the pyran ring was an essential requirement to observe cytotoxic activity in this series.[3,7–11] Based on those data, we formulated a hypothesis of bioactivation of acronycine by transformation of the 1,2-double bond into the corresponding epoxide *in vivo*[12] and synthesized a series of *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine diesters.[13,14] As expected, these compounds exhibited promising antitumor properties, with a broadened spectrum of activity and an increased potency when compared with acronycine itself on several tumor strains *in vitro* and *in vivo*.[13,14] In a continuation of our studies on structure–activity relationships in the acronycine series, and with the aim to clarify the role of the C-1 substitution, we report here the synthesis and cytotoxic activity of 1-alkoxy and 1-amino-2-hydroxy-1,2-dihydroacronycine derivatives.

The high reactivity of the benzylic position of *cis*-1,2-dihydroxy-1,2-dihydroacronycine (**2**) and its diacetate (**3**)[13] toward nucleophilic agents led us to consider these two compounds as suitable candidates for substitution reactions at C-1.

Treatment of *cis*-diol **2** with excess methanol in the presence of hydrochloric acid led to a 1 : 1 mixture of *cis*-2-hydroxy-1-methoxy-1,2-dihydroacronycine (**4**) and *trans*-2-hydroxy-1-methoxy-1,2-dihydroacronycine (**5**), which could be separated by column chromatography. These two methoxy compounds gave rise to the corresponding esters at the 2-position, **6**—**9**, upon treatment with acetic anhydride or chloroacetyl chloride in the presence of pyridine. It should be noted that *cis*-2-chloroacetoxy-1-methoxy-1,2-dihydroacronycine (**8**) is particularly unstable, most probably due to steric hindrance. For instance, it spontaneously reacts with trace amounts of water to give quantitatively alcohol **4**.

In a similar way, reaction of *cis*-1,2-dihydroxy-1,2-dihydroacronycine (**2**) with benzyl alcohol, carried out in tetrahydrofuran containing a catalytic amount of boron trifluoride, afforded a 3 : 7 isomeric mixture of *cis*-1-benzyloxy-2-hydroxy-1,2-dihydroacronycine (**10**) and *trans*-1-benzyloxy-2-hydroxy-1,2-dihydroacronycine (**11**). The solvent plays a crucial role in the course of this latter reaction. When dimethylformamide or acetonitrile are used instead of tetrahydrofuran, 2-oxo-1,2-dihydroacronycine (**12**) is the only product which can be isolated from the reaction mixture. Esterification reactions, using either acetic anhydride or chloroacetyl chloride could be successfully performed on the *trans*- isomer **11**, leading to *trans*-2-acetoxy-1-benzyloxy-1,2-dihydroacronycine (**13**) and to *trans*-1-benzyloxy-2-chloroacetoxy-1,2-dihydroacronycine (**14**), respectively. In contrast, acylation of the *cis*-isomer **10** seemed, like in the case of **4**, more difficult. Indeed, treatment of **10** with chloroacetyl chloride, even when the reaction was carried out at low temperature led to a complicated mixture from which only the unexpected *trans*-ester **14** could be isolated and identified (which probably results from epimerization at C-1).

Amination reactions at position-1 of the 1,2-dihydroacronycine system could be easily performed using *cis*-1,2-diacetoxy-1,2-dihydroacronycine (**3**).[13] Indeed, treatment of **3** with methylamine in ethanolic solution at reflux led to *trans*-2-acetoxy-1-methylamino-1,2-dihydroacronycine (**15**), accompanied by its saponification product *trans*-2-hydroxy-1-methylamino-1,2-dihydroacronycine (**16**). Treatment of the latter with excess acetic anhydride gave access to *trans*-2-acetoxy-1-methylacetamido-1,2-dihydroacronycine (**17**). Finally, reaction of **3** with hydrazine in ethanol gave *trans*-1-hydrazino-2-hydroxy-1,2-dihydroacronycine (**18**).

The formation of two epimeric ethers upon treatment of diol **2** by methanol or benzyl alcohol is a consequence of the *SN*1 type reaction previously known to occur at the benzylic position of various pyranocoumarins.[15,16] In contrast, amination of diester **3** proceeds with complete inversion of stereochemistry at C-1 *via* an *SN*2 type reaction. It is of interest to point out that treatment of diacetate **3** with excess of methanol gave the expected 1-alkoxy derivatives **6** and **7** in poor yield. The major compound of this reaction was 2-acetoxy-acronycine (**19**). The formation of compound **19**, in this case, could be explained by an elimination reaction *via* an E1 mechanism.

The study of the cytotoxic properties of the new acronycine derivatives was carried out *in vitro* on L-1210

* To whom correspondence should be addressed.

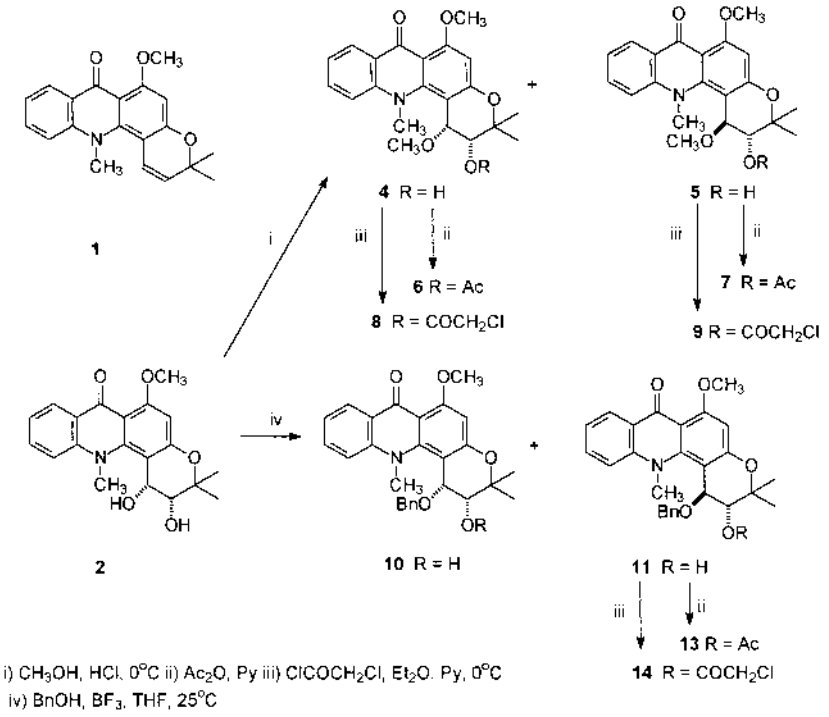

Chart 1

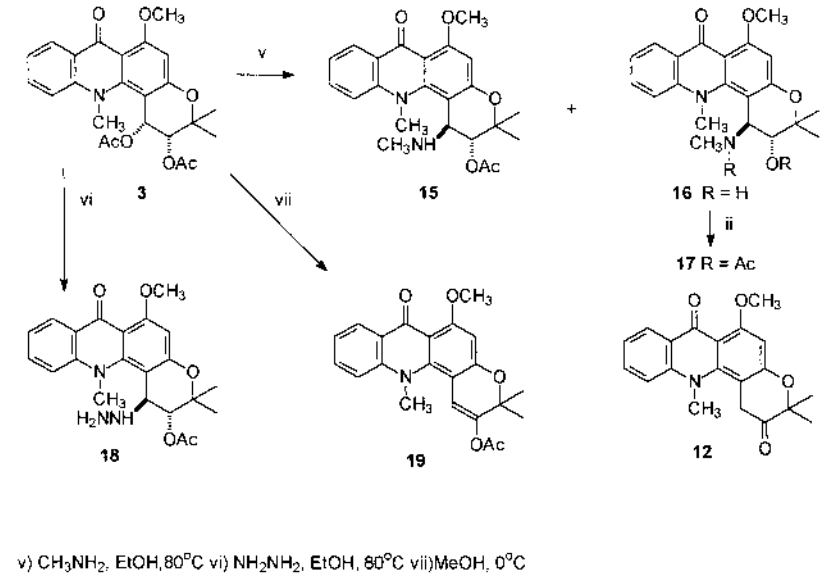

Chart 2

leukemia cells.[17,18] The results (IC_{50}) are summarized in Table 1. In contrast with *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine diesters, most 1-alkoxy and 1-amino-2-hydroxy-1,2-dihydroacronycine derivatives only exhibit marginal cytotoxic activity. This lack of significant activity confirms our previous hypothesis that cytotoxicity in this series is correlated with the presence of a good leaving group at the benzylic position, able to ensure sufficient reactivity toward nucleophilic agents.[13] It should nevertheless be noted that 1-alkoxy derivatives bearing a chloroacetyl ester group at position-2 such as **9** and **14** exhibit significant cytotoxic activity, within the same range of magnitude as the corresponding *cis* and *trans* diesters.[13,14]

Table 1. Cytotoxic Activity[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
4	44.6	**12**	43.3
5	50.1	**13**	>100
6	43.6	**14**	5.2
7	54.0	**15**	61.3
8	NT	**16**	56.6
9	14.7	**17**	75.8
10	40.5	**18**	>100
11	48.2	**19**	>100

a) Inhibition of L1210 cell proliferation measured by the MMT assay (mean of 2 values obtained in independent experiments). NT: non tested.

Experimental

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

(±)-*trans*-2-Hydroxy-1-methoxy-1,2-dihydroacronycine (5) To a solution of **2** (200 mg, 0.56 mmol) in MeOH (5 ml) was added HCl (10 N, 0.1 ml) and the reaction mixture was stirred for 48 h at 0 °C. Then the mixture was neutralized with resin IR-45 and the solvent was removed under reduced pressure. The remaining residue was purified by flash chromatography on Si gel with CH_2Cl_2–MeOH (99 : 1) to give compound **5** (60 mg, 29%) and **4** (60 mg, 29%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.36 (1H, dd, J=8, 1.5 Hz, H-8), 7.62 (1H, td, J=8, 1.5 Hz, H-10), 7.39 (1H, d, J=8 Hz, H-11), 7.24 (1H, t, J=8 Hz, H-9), 6.26 (1H, s, H-5), 4.97 (1H, d, J=8 Hz, H-1), 3.98 (3H, s, OMe-C6), 3.82 (1H, d, J=8 Hz, H-2), 3.81 (3H, s, NMe), 2.75 (3H, s, OMe-C1), 1.59 (3H, s, Me), 1.43 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.42 (C-7), 162.53 (C-6), 160.08 (C-4a), 148.21 (C-12a), 144.04 (C-11a), 132.48 (C-10), 126.89 (C-8), 125.17 (C-7a), 121.66 (C-9), 115.82 (C-11), 110.77 (C-6a), 99.17 (C-12b), 94.31 (C-5), 78.18 (C-3), 76.11 (C-1), 69.93 (C-2), 56.93 (OMe-C6), 49.92 (OMe-C1), 41.83 (NMe), 26.60 (Me), 17.48 (Me). MS-DCI *m/z*: 370 $(M+H)^+$.

(±)-*cis*-2-Hydroxy-1-methoxy-1,2-dihydroacronycine (4) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.36 (1H, dd, J=8, 1.5 Hz, H-8), 7.62 (1H, td, J=8, 1.5 Hz, H-10), 7.39 (1H, d, J=8 Hz, H-11), 7.24 (1H, t, J=8 Hz, H-9), 6.27 (1H, s, H-5), 4.80 (1H, d, J=4.6 Hz, H-1), 4.05 (1H, d, J=4.6 Hz, H-2), 3.97 (3H, s, OMe-C6), 3.62 (3H, s, NMe), 3.55 (3H, s, OMe-C1), 1.60 (3H, s, Me), 1.44 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.97 (C-7), 162.53 (C-6), 169.40 (C-4a), 149.32 (C-12a), 144.19 (C-11a), 132.47 (C-10), 127.01 (C-8), 125.25 (C-7a), 121.66 (C-9), 115.51 (C-11), 111.44 (C-6a), 98.14 (C-12b), 94.17 (C-5), 76.57 (C-3), 74.40 (C-1), 66.65 (C-2), 56.73 (OMe-C6), 55.45 (OMe-C1), 41.31 (NMe), 25.14 (Me), 21.73 (Me). MS-DCI *m/z*: 370 $(M+H)^+$.

(±)-*trans*-2-Acetoxy-1-methoxy-1,2-dihydroacronycine (7) To a solution of **5** (20 mg, 0.05 mmol) in dry pyridine (1.5 ml) was added Ac_2O (1.5 ml, 15 mmol). The reaction mixture was stirred for 24 h at room temperature and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Si gel with cyclohexane–EtOAc (50 : 50) to give compound **7** (19 mg, 85%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.33 (1H, dd, J=8, 1.5 Hz, H-8), 7.62 (1H, td, J=8, 1.5 Hz, H-10), 7.38 (1H, d, J=8 Hz, H-11), 7.25 (1H, t, J=8 Hz, H-9), 6.27 (1H, s, H-5), 5.30 (1H, d, J=9.5 Hz, H-2), 5.08 (1H, d, J=9.5 Hz, H-1), 3.99 (3H, s, OMe-C6), 3.82 (3H, s, NMe), 2.71 (3H, s, OMe-C1), 2.19 (3H, s, C$\underline{H}_3$CO), 1.46 (6H, s, 2Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.65 (C-7), 169.97 (CH$_3$$\underline{C}$O), 162.74 (C-6), 159.84 (C-4a), 148.37 (C-12a), 144.72 (C-11a), 132.71 (C-10), 126.89 (C-8), 125.58 (C-7a), 121.87 (C-9), 115.92 (C-11), 111.40 (C-6a), 98.90 (C-12b), 94.37 (C-5), 77.00 (C-3), 73.40 (C-1), 69.90 (C-2), 56.24 (OMe-C6), 50.49 (OMe-C1), 42.04 (NMe), 26.20 (Me), 21.02 ($\underline{C}$H$_3$CO), 18.93 (Me). MS-DCI *m/z*: 412 $(M+H)^+$.

(±)-*cis*-2-Acetoxy-1-methoxy-1,2-dihydroacronycine (6) Treatment of **4** under conditions essentially the same as those described for the preparation of **7** afforded compound **6**. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.34 (1H, dd, J=8, 1.5 Hz, H-8), 7.62 (1H, td, J=8, 1.5 Hz, H-10), 7.35 (1H, d, J=8 Hz, H-11), 7.26 (1H, t, J=8 Hz, H-9), 6.27 (1H, s, H-5), 5.52 (1H, d, J=5 Hz, H-2), 4.91 (1H, d, J=5 Hz, H-1), 3.99 (3H, s, OMe-C6), 3.73 (3H, s, NMe), 3.45 (3H, s, OMe-C1), 2.10 (3H, s, C$\underline{H}_3$CO), 1.53 (3H, s, Me), 1.42 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.60 (C-7), 171.12 (CH$_3$$\underline{C}$O), 162.70 (C-6), 158.80 (C-4a), 149.10 (C-12a), 144.41 (C-11a), 132.58 (C-10), 127.02 (C-8), 125.57 (C-7a), 121.72 (C-9), 115.72 (C-11), 111.90 (C-6a), 99.43 (C-12b), 94.26 (C-5), 77.00 (C-3), 73.04 (C-1), 68.44 (C-2), 56.44 (OMe-C6), 56.23 (OMe-C1), 41.50 (NMe), 25.25 (Me), 23.10 (Me), 20.96 ($\underline{C}$H$_3$CO). MS-DCI *m/z*: 412 $(M+H)^+$.

(±)-*trans*-2-Chloroacetoxy-1-methoxy-1,2-dihydroacronycine (9) To a solution of **5** (20 mg, 0.05 mmol) in dry Et_2O (5 ml) was added $ClCOCH_2Cl$ (0.25 ml) and dry pyridine (0.25 ml). The reaction mixture was stirred for 3 h at 0 °C and then the reagents were removed under reduced pressure (using a high vacuum pump). The residue was purified by flash chromatography on Si gel with cyclohexane–EtOAc (50 : 50) to give compound **9** (19 mg, 85%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.32 (1H, dd, J=8, 1.5 Hz, H-8), 7.62 (1H, td, J=8, 1.5 Hz, H-10), 7.35 (1H, d, J=8 Hz, H-11), 7.23 (1H, t, J=8 Hz, H-9), 6.25 (1H, s, H-5), 5.31 (1H, d, J=8 Hz, H-2), 5.08 (1H, d, J=8, 10 Hz, H-1), 4.17 (2H, s, ClC$\underline{H}_2$CO), 3.95 (3H, s, C6-OMe), 3.79 (3H, s, NMe), 2.65 (3H, s, C1-OMe), 1.46 (3H, s, Me), 1.45 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.60 (C-7), 165.80 (ClCH$_2$$\underline{C}$O), 162.80 (C-6), 159.83 (C-4a), 148.36 (C-12a), 144.69 (C-11a), 132.23 (C-10), 126.58 (C-8), 125.58 (C-7a), 121.62 (C-9), 115.57 (C-11), 111.43 (C-6a), 103.48 (C-12b), 94.00 (C-5), 78.97 (C-3), 73.07 (C-1), 71.55 (C-2), 56.02 (OMe), 50.27 (OMe-C1), 41.68 (NMe), 40.43 (Cl$\underline{C}$H$_2$CO), 25.86 (Me), 18.41 (Me). MS-DCI *m/z* : 448, 446 $(M+H)^+$.

(±)-*cis*-2-Chloroacetoxy-1-methoxy-1,2-dihydroacronycine (8) Treatment of **4** under conditions essentially the same as those described for the preparation of **9** afforded compound **8**. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.33 (1H, dd, J=8, 1.5 Hz, H-8), 7.60 (1H, td, J=8, 1.5 Hz, H-10), 7.31 (1H, d, J=8 Hz, H-11), 7.21 (1H, t, J=8 Hz, H-9), 6.22 (1H, s, H-5), 5.53 (1H, d, J=4.5 Hz, H-2), 4.88 (1H, d, J=4.5 Hz, H-1), 4.03 (2H, s, ClC$\underline{H}_2$CO), 3.96 (3H, s, C6-OMe), 3.70 (3H, s, NMe), 3.45 (3H, s, C1-OMe), 1.52 (3H, s, Me), 1.45 (3H, s, Me). MS-DCI *m/z*: 448, 446 $(M+H)^+$.

(±)-*trans*-1-Benzyloxy-2-hydroxy-1,2-dihydroacronycine (11) To a solution of **2** (100 mg, 0.28 mmol) in dry tetrahydrofuran (THF) (5 ml) was added benzyl alcohol (1 ml) and BF_3 ether complex (0.5 ml) and the reaction mixture was stirred for 5 d at room temperature. Then the mixture was neutralized with resin IR-45 and the solvent was removed under reduced pressure (using a high vacuum pump). The remaining residue was purified by flash chromatography on Si gel with CH_2Cl_2–MeOH (99 : 1) to give compound **11** (26 mg, 21%) and **10** (12 mg, 10%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.36 (1H, dd, J=8, 1.5 Hz, H-8), 7.50 (1H, td, J=8, 1.5 Hz, H-10), 7.20 (1H, t, J=8 Hz, H-9), 7.15 (1H, d, J=8 Hz, H-11), 7.02 (1H, t, J=8 Hz, H-4′), 7.00 (2H, t, J=8 Hz, H-3′, 5′), 6.82 (2H, d, J=8 Hz, H-2′, 6′), 6.27 (1H, s, H-5), 5.10 (1H, d, J=8 Hz, H-1), 4.12 (1H, d, J=12 Hz, C$\underline{H}_a$-C$_6$H$_5$), 3.99 (3H, s, OMe-C6), 3.98 (1H, d, J=8 Hz, H-2), 3.75 (3H, s, NMe), 3.62 (1H, d, J=12 Hz, C$\underline{H}_b$-C$_6$H$_5$), 1.59 (3H, s, Me), 1.45 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.59 (C-7), 162.78 (C-6), 160.16 (C-4a), 148.22 (C-12a), 144.51 (C-11a), 137.45 (C-1′), 132.52 (C-10), 128.15 (C-2′, 6′), 127.65 (C-3′, 5′), 127.47 (C-4′), 126.76 (C-8), 125.30 (C-7a), 121.72 (C-9), 116.02 (C-11), 111.00 (C-6a), 99.30 (C-12b), 94.34 (C-5), 78.17 (C-3), 76.60 (C-1), 71.02 (C-2), 65.23 ($\underline{C}$H$_2$-C$_6$H$_5$), 56.20 (OMe-C6), 41.91 (NMe), 26.68 (Me), 17.39 (Me). MS-DCI *m/z*: 446 $(M+H)^+$.

(±)-*cis*-1-Benzyloxy-2-hydroxy-1,2-dihydroacronycine (10) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.36 (1H, dd, J=8, 1.5 Hz, H-8), 7.60 (1H, td, J=8, 1.5 Hz, H-10), 7.24—7.20 (7H, m, H-9, 11, 2′, 3′, 4′, 5′, 6′), 6.27 (1H, s, H-5), 5.10 (1H, d, J=4.6 Hz, H-1), 4.90 (1H, d, J=12 Hz, C$\underline{H}_a$-C$_6$H$_5$), 4.71 (1H, d, J=12 Hz, C$\underline{H}_b$-C$_6$H$_5$), 4.15 (1H, d, J=8 Hz, H-2), 3.99 (3H, s, OMe-C6), 3.69 (3H, s, NMe), 1.62 (3H, s, Me), 1.50 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.50 (C-7), 162.70 (C-6), 159.61 (C-4a), 149.72 (C-12a), 144.29 (C-11a), 136.91 (C-1′), 132.33 (C-10), 128.61 (C-2′, 6′), 127.92 (C-4′), 126.99 (C-8), 126.69 (C-3′, 5′), 125.67 (C-7a), 121.76 (C-9), 115.94 (C-11), 112.00 (C-6a), 98.41 (C-12b), 94.34 (C-5), 76.79 (C-3), 73.74 (C-1), 69.80 ($\underline{C}$H$_2$-C$_6$H$_5$), 67.66 (C-2), 56.25 (OMe-C6), 41.89 (NMe), 25.23 (Me), 22.03 (Me). MS-DCI *m/z*: 446 $(M+H)^+$.

(±)-*trans*-2-Acetoxy-1-benzyloxy-1,2-dihydroacronycine (13) Treatment of **11** under conditions essentially the same as those described for the preparation of **7** afforded compound **13**. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.33 (1H, dd, J=8, 1.5 Hz, H-8), 7.54 (1H, td, J=8, 1.5 Hz, H-10), 7.20 (1H, t, J=8 Hz, H-9), 7.16 (1H, d, J=8 Hz, H-11), 7.09 (1H, t, J=8 Hz, H-4′), 7.00 (2H, t, J=8 Hz, H-3′, 5′), 6.77 (2H, d, J=8 Hz, H-2′, 6′), 6.29 (1H, s, H-5), 5.48 (1H, d, J=8 Hz, H-2), 5.21 (1H, d, J=8 Hz, H-1), 4.18 (1H, d, J=9 Hz, C$\underline{H}_a$-C$_6$H$_5$), 3.98 (3H, s, OMe-C6), 3.75 (3H, s, NMe), 3.37 (1H, d, J=9 Hz, C$\underline{H}_b$-C$_6$H$_5$), 1.48 (3H, s, Me), 1.46 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.62 (C-7), 169.98 ($\underline{C}$OCH$_3$), 162.87 (C-6), 159.83 (C-4a), 148.20 (C-12a), 144.60 (C-11a), 136.92 (C-1′), 132.47 (C-10), 128.58 (C-2′, 6′), 128.04 (C-3′, 5′), 127.49 (C-4′), 126.82 (C-8), 125.49 (C-7a), 121.84 (C-9), 116.13 (C-11), 111.43 (C-6a), 99.26 (C-12b), 94.38 (C-5), 77.00 (C-3), 73.67 (C-1), 70.48 (C-2), 65.39 ($\underline{C}$H$_2$-C$_6$H$_5$), 56.26 (OMe-C6), 41.98 (NMe), 26.26 (Me), 21.04 (CO$\underline{C}$H$_3$), 18.65 (Me). MS-DCI *m/z*: 446 $(M+H)^+$.

(±)-*trans*-2-Chloroacetoxy-1-benzyloxy-1,2-dihydroacronycine (14) Treatment of **11** under conditions essentially the same as those described for the preparation of **9** afforded compound **14**. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.36 (1H, dd, J=8, 1.5 Hz, H-8), 7.58 (1H, td, J=8, 1.5 Hz, H-10), 7.24 (1H, t, J=8 Hz, H-9), 7.13 (1H, d, J=8 Hz, H-11), 7.00 (1H, t, J=8 Hz, H-4′),

7.00 (2H, t, J=8 Hz, H-3′, 5′), 6.72 (2H, d, J=8 Hz, H-2′, 6′), 6.35 (1H, s, H-5), 5.55 (1H, d, J=8 Hz, H-2), 5.28 (1H, d, J=8 Hz, H-1), 4.20 (1H, d, J=9 Hz, C$\underline{H}_a$-C_6H_5), 4.20 (2H, s, COC$\underline{H}_2$Cl), 4.02 (3H, s, OMe-C6), 3.80 (3H, s, NMe), 3.52 (1H, d, J=9 Hz, C$\underline{H}_b$-C_6H_5), 1.52 (3H, s, Me), 1.51 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.59 (C-7), 166.59 (C$\underline{O}$CH$_2$Cl), 16290 (C-6), 159.62 (C-4a), 148.17 (C-12a), 144.55 (C-11a), 136.66 (C-1′), 132.55 (C-10), 128.60 (C-2′, 6′), 128.09 (C-3′, 5′), 127.61 (C-4′), 126.83 (C-8), 126.50 (C-7a), 121.95 (C-9), 116.14 (C-11), 111.00 (C-6a), 98.83 (C-12b), 94.37 (C-5), 76.90 (C-3), 73.54 (C-1), 72.51 (C-2), 65.38 (C$\underline{H}_2$-C_6H_5), 56.31 (OMe-C6), 41.96 (NMe), 40.73 (CO$\underline{C}$H$_2$Cl), 26.26 (Me), 21.04 (CO$\underline{C}$H$_3$), 18.53 (Me). MS-DCI *m/z*: 512 $(M+H)^+$.

(±)-*trans*-2-Hydroxy-1-methylamino-1,2-dihydroacronycine (16) To a solution of **3** (62 mg, 0.14 mmol) in EtOH (2 ml) was added CH_3NH_2 (0.25 ml, 40% soln. in water). The reaction mixture was stirred for 16 h at 80 °C and then the reagents were removed under reduced pressure. The remaining residue was purified by flash chromatography on Si gel with CH_2Cl_2–MeOH (99 : 1) to give compound **16** (20 mg, 38%) and **14** (6 mg, 10%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.32 (1H, dd, J=8, 1.5 Hz, H-8), 7.60 (1H, td, J=8, 1.5 Hz, H-10), 7.31 (1H, d, J=8 Hz, H-11), 7.22 (1H, t, J=8 Hz, H-9), 6.23 (1H, s, H-5), 4.02 (1H, d, J=8 Hz, H-2), 3.93 (3H, s, OMe-C6), 3.69 (3H, s, NMe), 3.59 (1H, d, J=8 Hz, H-1), 1.61 (3H, s, NMe-C1), 1.54 (3H, s, Me), 1.38 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.52 (C-7), 161.82 (C-6), 160.14 (C-4a), 148.99 (C-12a), 144.97 (C-11a), 132.71 (C-10), 127.11 (C-8), 125.53 (C-7a), 122.06 (C-9), 116.18 (C-11), 111.00 (C-6a), 100.92 (C-12b), 94.80 (C-5), 77.00 (C-3), 70.71 (C-2), 57.66 (C-1), 56.14 (OMe-C6), 43.29 (NMe), 28.15 (NMe-C1), 26.70 (Me), 16.98 (Me). MS-DCI *m/z*: 369 $(M+H)^+$.

(±)-*trans*-2-Acetoxy-1-methylamino-1,2-dihydroacronycine (15) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.32 (1H, dd, J=8, 1.5 Hz, H-8), 7.61 (1H, td, J=8, 1.5 Hz, H-10), 7.23 (1H, d, J=8 Hz, H-11), 7.23 (1H, t, J=8 Hz, H-9), 6.28 (1H, s, H-5), 5.98 (1H, d, J=8 Hz, H-2), 4.11 (1H, d, J=3 Hz, N-H), 3.95 (3H, s, OMe-C6), 3.63 (1H, dd, J=8, 3 Hz, H-1), 3.61 (3H, s, NMe), 2.71 (3H, s, OMe-C1), 2.01 (3H, s, C$\underline{H}_3$CO), 1.99 (3H, s, NMe-C1), 1.54 (3H, s, Me), 1.52 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.34 (C-7), 175.51 (CH$_3\underline{C}$O), 162.55 (C-6), 160.44 (C-4a), 148.58 (C-12a), 144.97 (C-11a), 133.08 (C-10), 127.19 (C-8), 125.53 (C-7a), 122.25 (C-9), 115.69 (C-11), 110.00 (C-6a), 98.55 (C-12b), 94.99 (C-5), 78.10 (C-3), 75.38 (C-2), 56.28 (OMe-C6), 55.71 (C-1), 42.66 (NMe), 31.34 (NMe-C1), 26.12 (Me), 22.52 (C$\underline{H}_3$CO), 16.80 (Me). MS-DCI *m/z*: 411 $(M+H)^+$.

(±)-*trans*-2-Acetoxy-1-methylacetamido-1,2-dihydroacronycine (17) Treatment of **16** under conditions essentially the same as those described for the preparation of **7** afforded compound **17**. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.30 (1H, dd, J=8, 1.5 Hz, H-8), 7.58 (1H, td, J=8, 1.5 Hz, H-10), 7.20 (1H, d, J=8 Hz, H-11), 7.20 (1H, t, J=8 Hz, H-9), 6.28 (1H, s, H-5), 6.20 (1H, d, J=8 Hz, H-1), 4.93 (1H, d, J=8 Hz, H-2), 3.96 (3H, s, OMe-C6), 3.63 (3H, s, NMe), 2.10 (3H, s, CH_3COO-C_2), 2.03 (3H, s, NMe-C1), 1.89 (3H, s, CH_3CON-C1), 1.58 (3H, s, Me), 1.41 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.33 (C-7), 171.69 (CH$_3\underline{C}$ON-C1), 170.73 (CH$_3\underline{C}$OO-C2), 162.50 (C-6), 160.14 (C-4a), 148.74 (C-12a), 144.99 (C-11a), 132.96 (C-10), 127.04 (C-8), 125.44 (C-7a), 122.13 (C-9), 116.10 (C-11), 112.00 (C-6a), 99.35 (C-12b), 94.55 (C-5), 77.00 (C-3), 72.78 (C-2), 56.27 (OMe-C6), 52.67 (C-1), 42.97 (NMe), 30.83 (NMe-C1), 26.06 (Me), 22.60 (C$\underline{H}_3$CON-C1), 20.99 (C$\underline{H}_3$COO-C2), 18.15 (Me). MS-DCI *m/z*: 453 $(M+H)^+$.

(±)-*trans*-2-Acetoxy-1-hydrazino-1,2-dihydroacronycine (18) To a solution of **3** (55 mg, 0.13 mmol) in EtOH (2 ml) was added NH_2NH_2 hydrate (0.2 ml). The reaction mixture was stirred for 3 h at 80 °C and then the reagents were removed under reduced pressure. Compound **18** was purified by crystallization with MeOH (21 mg, 40%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.90 (1H, br, N-H), 7.71 (1H, dd, J=8, 1.5 Hz, H-8), 7.55 (1H, td, J=8, 1.5 Hz, H-10), 7.33 (1H, d, J=8 Hz, H-11), 6.96 (1H, t, J=8 Hz, H-9), 5.90 (1H, s, H-5), 5.47 (1H, br, N-H), 5.12 (1H, br, N-H),4.12 (1H, d, J=8.8 Hz, H-2), 3.72 (3H, s, OMe-C6), 3.48 (1H, d, J=8.8 Hz, H-1), 3.45 (3H, s, NMe), 1.90 (3H, s, C$\underline{H}_3$CO), 1.60 (3H, s, Me), 1.38 (3H, s, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.03 (C-7), 170.64 (CH$_3\underline{C}$O), 161.96 (C-6), 160.20 (C-4a), 149.24 (C-12a), 145.05 (C-11a), 133.51 (C-10), 126.24 (C-8), 125.06 (C-7a), 122.05 (C-9), 117.79 (C-11), 110.44 (C-6a), 101.61 (C-12b), 94.90 (C-5), 79.49 (C-3), 71.49 (C-2), 59.78 (C-1), 56.31 (OMe-C6), 43.40 (NMe), 27.20 (Me), 20.76 (C$\underline{H}_3$CO), 18.24 (Me). MS-DCI *m/z*: 412 $(M+H)^+$.

2-Acetoxy-acronycine (19) A solution of **3** (55 mg, 0.13 mmol) in MeOH (5 ml) was stirred for 48 h at 0 °C. The solvent was removed under reduced pressure and the remaining residue was purified by flash chromatography on Si gel with CH_2Cl_2–MeOH (99.5 : 0.5) to give compound **19** (24 mg, 50%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 8.39 (1H, dd, J=8, 1.5 Hz, H-8), 7.63 (1H, td, J=8, 1.5 Hz, H-10), 7.34 (1H, d, J=8 Hz, H-11), 7.23 (1H, t, J=8 Hz, H-9), 6.50 (1H, s, H-1), 6.38 (1H, s, H-5), 3.99 (3H, s, OMe-C6), 3.85 (3H, s, NMe), 2.27 (3H, s, C$\underline{H}_3$CO), 1.56 (3H, s, 2×Me). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 177.38 (C-7), 169.25 (CH$_3\underline{C}$O), 162.79 (C-6), 157.14 (C-4a), 146.57 (C-12a), 144.57 (C-11a), 140.86 (C-2), 132.72 (C-10), 127.24 (C-8), 125.48 (C-7a), 121.96 (C-9), 115.89 (C-11), 111.07 (C-6a), 109.82 (C-1), 102.18 (C-12b), 94.14 (C-5), 77.00 (C-3), 56.34 (OMe-C6), 44.23 (NMe), 24.04 (2×Me), 21.07 (C$\underline{H}_3$CO). MS-DCI *m/z*: 380 $(M+H)^+$.

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.[17] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC_{50}, 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) Hughes G. K., Lahey F. N., Price J. R., Webb L. J., *Nature* (London), **62**, 223—224 (1948).
2) Macdonald P. L., Robertson A. V., *Aust. J. Chem.*, **19**, 275—281 (1966).
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) Dorr T. R., Liddil J. D., Von Hoff D. D., Soble M., Osborne C. K., *Cancer Res.*, **49**, 340—344 (1989).
6) Scarffe H. J., Beaumont A. R., Crowther D., *Cancer Treatment Reports*, **67**, 93—94 (1983).
7) Schneider J., Evans E. L., Grunberg E., Fryer R. I., *J. Med. Chem.*, **15**, 266—270 (1972).
8) Shieh H. L., Pezzuto J. M., Cordell G. A., *Chem.-Biol. Interact*., **81**, 35—55 (1992).
9) Elomri A., Michel S., Tillequin F., Koch M., *Heterocycles*, **34**, 799—806 (1992).
10) Mitaku S., Skaltsounis A. L., Tillequin F., Koch M., Rolland Y., Pierre A., Atassi Gh., *Pharm. Res*., **13**, 939—943 (1996).
11) Elomri A., Skaltsounis A. L., Michel S., Tillequin F., Koch M., Rolland Y., Pierre A., Atassi Gh., *Chem. Pharm. Bull.*, **44**, 2165—2168 (1996).
12) Brum-Bousquet M., Mitaku S., Skaltsounis A. L., Tillequin F., Koch M., *Planta Med*., **54**, 470—471 (1988).
13) Elomri A., Mitaku S., Michel S., Skaltsounis A. L., Tillequin F., Koch M., Pierre A., Guilbaud N., Léonce S., Kraus-Berthier L., Rolland Y., Atassi Gh., *J. Med. Chem*., **39**, 4762—4766 (1996).
14) Magiatis P., Mitaku S., Skaltsounis A. L., Tillequin F., Koch M., Pierré A., Atassi Gh., *J. Nat. Prod*., **61**, 198—201 (1998).
15) Shnoder H. D., Bencze W., Halpern O., Schmid H., *Chem. Ber.*, **92**, 2338—2363 (1959).
16) Lemmich J., Lemmich E., Nielsen B. E., *Acta Chem. Scand*., **20**, 2497—2507 (1966).
17) Pierré A., Dunn T. A., Kraus-Berthier L., Léonce S., Saint-Dizier D., Regnier G., Dhainaut A., Berlion M., Bizzari J. P., Atassi Gh., *Invest. New Drug*, **10**, 137—148 (1992).
18) Léonce S., Perez V., Casabianca-Pignede M. R., Anstett M., Bisagni E., Pierré A., Atassi Gh., *Invest. New Drug*, **14**, 169—180 (1996).