Design, Synthesis, and Cytotoxic Activity of Michael Acceptors and Enol Esters in the Benzo[*b*]acronycine Series

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A series of 2-acyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones (4—6) was prepared by treatment of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (3) with an excess of an appropriate acyl chloride in the presence of aluminum chloride. Treatment of (±)-*cis*-1-hydroxy-2-acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones (9, 10) or (±)-*cis*-1,2-diacyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones (2, 11) with hydrochloric acid gave the corresponding 2-acyloxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones, exemplified by acetate 7 and butyrate 8. None of the Michael acceptors 4—6 showed significant antiproliferative activity. Enol esters 7 and 8 were markedly cytotoxic toward L1210 leukemia cells, with IC_{50} values within the same range of magnitude as (±)-*cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (S23906-1), currently under phase I clinical trials. In contrast with S23906-1, enol esters 7 and 8 were not reactive toward purified DNA.

Key words acronycine; benzo[*b*]acronycine; cytotoxicity

The pyranoacridone alkaloid acronycine (**1**), originally isolated from *Acronychia baueri* Schott (Rutaceae)[1—3] has shown antitumor properties in a panel of murine solid tumor models, including S-180 and AKR sarcomas, X-5563 myeloma, S-115 carcinoma, and S-91 melanoma.[4,5] However, its moderate potency and poor solubility in aqueous solvents severely hampered the subsequent clinical trials, which were rapidly discontinued, due to modest therapeutic effects and dose-limiting gastrointestinal toxicity after oral administration.[6] Consequently, the development of structural analogues with increased potency and/or better water solubility was highly desirable.

Our efforts toward the obtainment of more potent derivatives were guided by a hypothesis of bioactivation of the 1,2-double bond of acronycine into the corresponding epoxide *in vivo*.[7] Significant improvements in terms of solubility and potency were obtained with derivatives modified in the pyran ring, which had a similar reactivity toward nucleophilic agents as acronycine epoxide, but an improved chemical stability. Such compounds are exemplified by diesters of *cis*-1,2-dihydroxy-1,2-dihydroacronycine[8] and diesters of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine ((±)-*cis*-1,2-dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one).[9] Representatives of this latter series are considered as valuable drug candidates for clinical studies.[10] For instance, diacetate **2**, currently developed under the code S23906-1 is currently under phase I clinical trials. Their mechanism of action implies alkylation of the 2-amino group of DNA guanine residues by the carbocation resulting from the elimination of the ester leaving group at position 1 of the drug.[11—14]

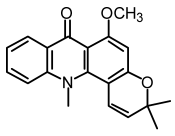

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Chart 1

In the course of the exploration of the structure activity relationships in the acronycine series,[15] 2-nitroacronycine, although shown to be toxic *in vivo*, demonstrated remarkably high potency when tested against a battery of cultured mammalian cells *in vitro*.[4,16,17] In this context, Michael acceptors in the benzo[*b*]acronycine series, possessing a 1,2-double bond and substituted at position 2 by an acyl group appeared as possible new drug candidates able to undergo additions at position 1 onto intracellular nuclophilic targets. Additionally, enol esters at position 2 were also worth exploring for comparison purposes. We describe here the synthesis and biological activities of various 2-acyl- and 2-acyloxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones.

Chemistry

Treatment of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**3**) with an excess of an appropriate acyl chloride in the presence of aluminum chloride in dichloromethane afforded the corresponding 2-acyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo-[*b*]pyrano[3,2-*h*]acridin-7-ones. Following this procedure, the desired 2-acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**), 2-butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one (**5**), and 2-benzoyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**6**) were prepared in 96, 54, and 42% yield, respectively.

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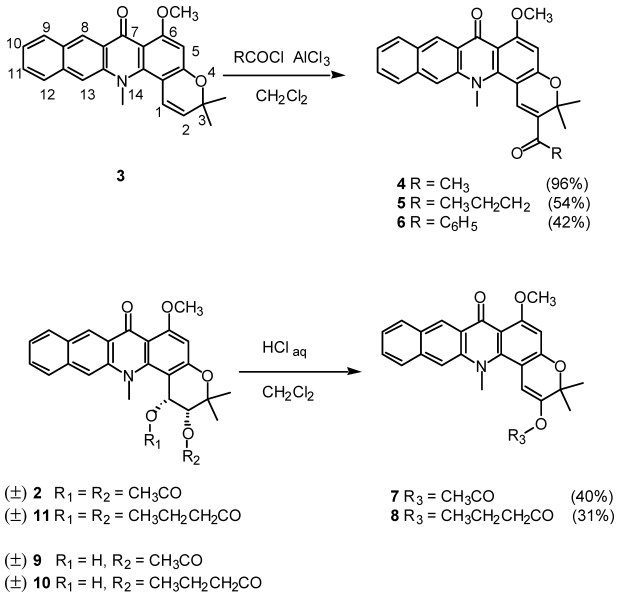

Chart 2

Enol esters at position 2, exemplified by acetate **7** and butyrate **8**, were obtained in moderate 40 and 31% yield, respectively, by dehydration of their corresponding (±)-*cis*-1-hydroxy-2-acyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones **9** and **10** with hydrochloric acid in dichloromethane. When the same reaction was applied to (±)-*cis*-1,2-diacyloxy-6-methoxy-3,-3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-ones **2** and **11**, enol esters **7** and **8** were also obtained, but in lower yields (19, 12%, respectively) than when the monoesters **9** and **10** were used as starting materials.

Pharmacology

The study of the biological properties of the new 2-acylbenzo[*b*]acronycines **4**, **5**, and **6**, and of the enol esters **7** and **8** was carried out *in vitro* in the L1210 murine leukemia cell line. The results (IC_{50} values) are reported in Table 1. None of the Michael acceptors **4**—**6** showed significant antiproliferative activity. In contrast, enol esters **7** and **8** were markedly cytotoxic, with IC_{50} values within the same range of magnitude as diacetate **2**, currently under phase I clinical trials.

The perturbation of the cell cycle induced by the active enol esters **7** and **8** was studied in the same cell line. Gel retardation experiments with DNA fragments were also performed for these two compounds, in comparison with diacetate **2** previously shown to alkylate purified DNA at the N-2 of guanine residues.[11,12] The results are summarized in Table 2. As previously established, diacetate **2** induced a partially reversible accumulation in the G2+M phases of the cell cycle at low concentration and an irreversible arrest in the S phase at higher concentration, whereas accumulation in the G1 phase was observed with enol acetate **7**. No alkylation of purified DNA was observed with both compounds **7** and **8**. At the cellular level, the mechanism of action of the compound **8** is different from that of **2**. Indeed, compound **8** did not induce an S phase accumulation, which is a common characteristic of all the derivatives acting as compound **2**. Compound **8** only induced a partial accumulation in the G2+M phases. The mechanism of action of compound **7** is clearly different from that of compounds **2** and **8**, as shown by a completely different perturbation of the cell cycle, *i.e.* slowing down during the G1 phase.

Table 1. Inhibition of L1210 Cell Proliferation by Compounds **4**—**8** in Comparison with Benzo[*b*]acronycine (**3**) and S23906-1 (**2**)

Compound	**2**	**3**	**4**	**5**	**6**	**7**	**8**
IC_{50} (μM)	0.8	15	20	30	>50	0.75	1.8

Table 2. Cell Cycle Perturbation Induced in L1210 Cell Line and *in Vitro* DNA Alkylation Induced by Compounds **7** and **8** in Comparison with S23906-1 (**2**)

Compound	**2**	**7**	**8**
Cell cycle perturbation	72% G2+M (1 μM) 73% S (5 μM)	55% G1 (20 μM)	51% G2+M (20 μM)
In vitro DNA Alkylation	++	0	0

The capacity of the tested compounds to form complexes with purified DNA was investigated by gel shift assay. Symbol ++ refers to strong alkylation, whereas 0 means no alkylation at all.

Results and Discussion

Considering the structure activity relationships in the benzo[*b*]acronycine series, it appears that compounds bearing an acyl substituent at position 2, initially considered as Michael acceptors able to undergo additions to nucleophilic cellular targets, were devoid of cytotoxic activity. From a chemical point of view, this result should be correlated with the high delocalization of the electrons in the benzo[*b*]-acronycine chromophore.

Enol esters at position 2, exemplified by **7** and **8**, displayed cytotoxic activities within the same range of magnitude as (±)-*cis*-1,2-diacetoxy-1,2-dihydro benzo[*b*]acronycine. Nevertheless, the mechanism of their action at the molecular level differs from that of **2**. In agreement with this statement, both enol esters **7** and **8** did not bind to purified DNA.

Experimental

Chemistry The melting points were determined on a Leica VM apparatus and are not corrected. IR spectra (ν_{max} in cm^{-1}) were obtained on a Perkin-Elmer 257 instrument. UV spectra (λ_{max} in nm) were determined in spectroscopic-grade MeOH on a Beckman Model 34 spectrophotometer. ^{1}H-NMR (δ [ppm], J [Hz]) and ^{13}C-NMR spectra were recorded at 400 and 100 MHz respectively, using a Bruker Avance 400 spectrometer. When necessary, the signals were unambiguously assigned by 2D NMR techniques: ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HMQC, and ^{13}C–^{1}H HMBC. These experiments were performed using standard Bruker microprograms. Mass spectra were recorded with a Nermag R-10-10C spectrometer using electron impact ionization (EI-MS; 70 eV) technique. Flash column chromatographies were performed using silica gel 60 Merck (35—70 mm) with an overpressure of 300 mbar.

2-Acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one (4) Acetyl chloride (59 μl, 0.824 mmol) and aluminium chloride (108 mg, 0.673 mmol) were added to a solution of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**3**) (50 mg, 0.1374 mmol) in CH_2Cl_2 (2 ml) at 0 °C. The reaction mixture was stirred at 20 °C for 3 h and poured onto 10% aqueous HCl solution (100 ml) at 0 °C. The mixture was extracted with CH_2Cl_2 (3×100 ml). The combined organic layer was washed with saturated Na_2CO_3 aqueous solution (100 ml), water (2×100 ml), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99 : 1 to 95 : 5) gave **4** (54 mg, 96%) as yellow needles, mp

277 °C (EtOH). IR (KBr) cm^{-1}: 3050, 2973, 2940, 1647, 1602, 1559, 1488, 1459, 1405, 1364, 1238, 1209, 1123, 1087, 809, 735. UV λ_{max} (MeOH) nm (log ε): 238 (4.36), 271 (4.76), 339 (4.58), 440 (3.85). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.94 (1H, s, C8-H), 8.05 (1H, dd, J=9, 1 Hz, C9-H), 7.89 (1H, dd, J=9, 1 Hz, C12-H), 7.73 (1H, s, C13-H), 7.59 (1H, ddd, J=9, 8, 1 Hz, C11-H), 7.55 (1H, s, C1-H), 7.47 (1H, ddd, J=9, 8, 1 Hz, C10-H), 6.29 (1H, s, C5-H), 4.03 (3H, s, O-C$\underline{H}_3$), 3.99 (3H, s, N-C$\underline{H}_3$), 2.47 (3H, s, C2-COC$\underline{H}_3$), 1.72 (6H, s, C3-(C$\underline{H}_3$)$_2$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 195.2 (C2-$\underline{C}$O–), 177.5 (C-7), 165.9 (C-6), 161.3 (C-4a), 148.4 (C-14a), 142.1 (C-13a), 135.7 (C-12a), 132.6 (C-2), 131.5 (C-1), 129.6 (C-9), 129.0 (C-8a), 128.5 (C-11), 128.3 (C-8), 126.8 (C-12), 125.4 (C-7a), 124.9 (C-10), 112.4 (C-13), 109.6 (C-6a), 102.4 (C-14b), 93.7 (C-5), 79.9 (C-3), 56.6 (O$\underline{C}$H$_3$), 45.4 (N$\underline{C}$H$_3$), 26.6 (C2-CO$\underline{C}$H$_3$), 25.9 (C3($\underline{C}$H$_3$)$_2$). EI-MS m/z: 413 $[M]^{+\cdot}$, 398 $[M-CH_3]^+$. *Anal.* Calcd for $C_{26}H_{23}NO_4$: C, 75.53; H, 5.61; N, 3.39. Found: C, 75.22; H, 5.67; N, 3.46.

2-Butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (5) Compound **5** was obtained from **3** (50 mg, 0.137 mmol) under conditions similar with those described for the preparation of **4**, using butyryl chloride (84 μl, 0.808 mmol) instead of acetyl chloride. Purification by flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99 : 1 to 94 : 6) gave **5** (32 mg, 54%) as pale yellow needles, mp 302 °C (acetone/hexane 7 : 3). IR (KBr) cm^{-1}: 3050, 2966, 2870, 1636, 1592, 1577, 1563, 1489, 1462, 1404, 1360, 1246, 1211, 1172, 1125, 1087, 1028, 804, 738. UV λ_{max} (MeOH) nm (log ε): 237 (4.40), 271 (4.79), 339 (4.61), 442 (3.87). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.94 (1H, s, C8-H), 8.05 (1H, dd, J=9, 1 Hz, C9-H), 7.89 (1H, dd, J=9, 1 Hz, C12-H), 7.74 (1H, s, C13-H), 7.57 (1H, ddd, J=9, 8, 1 Hz, C11-H), 7.50 (1H, s, C1-H), 7.45 (1H, ddd, J=9, 8, 1 Hz, C10-H), 6.29 (1H, s, C5-H), 4.04 (3H, s, O-C$\underline{H}_3$), 3.97 (3H, s, N-C$\underline{H}_3$), 2.76 (2H, t, J=7.5 Hz, C2-COC$\underline{H}_2$CH$_2$CH$_3$), 1.75 (2H, m, C2-COCH$_2$C$\underline{H}_2$CH$_3$), 1.72 (6H, s, C3-(C$\underline{H}_3$)$_2$), 1.02 (3H, t, J=7.5 Hz, C2-COCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 198.1 (C2-$\underline{C}$O–), 177.6 (C-7), 165.7 (C-6), 161.2 (C-4a), 148.8 (C-14a), 141.5 (C-13a), 135.7 (C-12a), 131.6 (C-2), 131.1 (C-1), 129.7 (C-9), 128.9 (C-8a), 128.5 (C-11), 128.4 (C-8), 126.8 (C-12), 125.4 (C-7a), 124.9 (C-10), 112.4 (C-13), 110.0 (C-6a), 102.1 (C-14b), 93.7 (C-5), 79.9 (C-3), 56.6 (O$\underline{C}$H$_3$), 45.4 (N$\underline{C}$H$_3$), 40.3 (C2-CO$\underline{C}$H$_2$CH$_2$CH$_3$), 25.9 (C3($\underline{C}$H$_3$)$_2$), 18.6 (C2-COCH$_2$$\underline{C}H_2CH_3$), 13.9 (C2-COCH$_2CH_2$$\underline{C}H_3$). EI-MS m/z: 441 $[M]^{+\cdot}$, 426 $[M-CH_3]^+$, 412 $[M-C_2H_5]^+$. *Anal.* Calcd for $C_{28}H_{27}NO_4$: C, 76.17; H, 6.16; N, 3.17. Found: C, 76.21; H, 6.07; N, 3.26.

2-Benzoyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (6) Compound **6** was obtained from **3** (50 mg, 0.137 mmol) under conditions similar with those described for the preparation of **4**, using benzoyl chloride (384 μl, 3.23 mmol) instead of acetyl chloride. Purification by flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99 : 1 to 94 : 6) gave **6** (27 mg, 42%) as an orange amorphous solid. IR (KBr) cm^{-1}: 3050, 2966, 2929, 1632, 1510, 1585, 1559, 1489, 1459, 1403, 1360, 1246, 1213, 1135, 1084, 1025, 805, 729. UV λ_{max} (MeOH) nm (log ε): 240 (4.37), 271 (4.62), 343 (4.42), 437 (4.14). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.94 (1H, s, C8-H), 8.03 (1H, d, J=9 Hz, C9-H), 7.80 (3H, m, C12-H, C2′-H, C6′-H), 7.57 (3H, m, C13-H, C11-H, C4′-H), 7.48 (3H, m, C10-H, C3′-H, C5′-H), 7.10 (1H, s, C1-H), 6.36 (1H, s, C5-H), 4.07 (3H, s, O-C$\underline{H}_3$), 3.73 (3H, s, N-C$\underline{H}_3$), 1.82 (6H, s, C3(C$\underline{H}_3$)$_2$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 193.9 (C2-$\underline{C}$O–), 178.0 (C-7), 165.6 (C-6), 161.3 (C-4a), 148.6 (C-14a), 140.9 (C-13a), 137.9 (C-12a), 135.6 (C-2), 134.0 (C-1), 132.4 (C-4′), 130.5 (C-1′), 129.4 (C-2′+C-6′), 129.3 (C-9), 128.7 (C-8a), 128.4 (C-11+C-3′+C-5′), 128.0 (C-8), 126.7 (C-12), 124.9 (C-10), 124.7 (C-7a), 112.4 (C-13), 109.1 (C-6a), 102.2 (C-14b), 93.6 (C-5), 79.8 (C-3), 56.2 (O$\underline{C}$H$_3$), 44.8 (N$\underline{C}$H$_3$), 25.2 (C3($\underline{C}$H$_3$)$_2$). EI-MS m/z: 475 $[M]^{+\cdot}$, 460 $[M-CH_3]^+$. *Anal.* Calcd for $C_{31}H_{25}NO_4$: C, 78.30; H, 5.30; N, 2.95. Found: C, 78.20; H, 5.37; N, 2.96.

2-Acetoxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (7) Method a: Aqueous HCl solution (10%, 0.2 ml) was added to a solution of (±)-*cis*-2-acetoxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**9**) (40 mg, 0.082 mmol) in CH_2Cl_2 (2 ml). The reaction mixture was stirred for 4 d at 20 °C and evaporated under reduced pressure. Flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99 : 1 to 90 : 10) gave **7** (14 mg, 40%) as orange needles, mp 165 °C (acetone). IR (KBr) cm^{-1}: 3048, 2971, 2931, 1765, 1647, 1620, 1586, 1569, 1492, 1461, 1397, 1333, 1231, 1194, 1110, 1086, 1029, 874, 799. UV λ_{max} (MeOH) nm (log ε): 240 (4.37), 275 (4.72), 307 (4.70), 360 (3.93), 443 (3.71). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.90 (1H, s, C8-H), 8.02 (1H, dd, J=9, 1 Hz, C9-H), 7.87 (1H, dd, J=9, 1 Hz, C12-H), 7.65 (1H, s, C13-H), 7.54 (1H, ddd, J=9, 8, 1 Hz, C11-H), 7.41 (1H, ddd, J=9, 8, 1 Hz, C10-H), 6.54 (1H, s, C1-H), 6.35 (1H, s, C5-H), 4.01 (3H, s, O-C$\underline{H}_3$), 3.95 (3H, s, N-C$\underline{H}_3$), 2.29 (3H, s, C2-OCOC$\underline{H}_3$), 1.59 (6H, s, C3-(C$\underline{H}_3$)$_2$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 178.2 (C-7), 169.2 (C2-O$\underline{C}$OCH$_3$), 163.0 (C-6), 157.7 (C-4a), 150.1 (C-14a), 142.0 (C-2), 141.0 (C-13a), 135.8 (C-12a), 129.6 (C-9), 128.7 (C-8a), 128.2 (C-11+C-8), 126.8 (C-12), 125.5 (C-7a), 124.5 (C-10), 112.5 (C-6a), 111.9 (C-13), 109.9 (C-5), 102.3 (C-14b), 93.8 (C-1), 78.0 (C-3), 56.4 (O$\underline{C}$H$_3$), 44.7 (N$\underline{C}$H$_3$), 24.1 (C3($\underline{C}$H$_3$)$_2$), 21.1 (C2-OCO$\underline{C}$H$_3$). EI-MS m/z: 429 $[M]^{+\cdot}$, 387 $[M-COCH_2]^{+\cdot}$. *Anal.* Calcd for $C_{26}H_{23}NO_5$: C, 72.71; H, 5.40; N, 3.26. Found: C, 77.81; H, 5.45; N, 3.21.

Method b: The same reaction applied to (±)-*cis*-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**2**) (40 mg, 0.0818 mmol) afforded **7** (11 mg, 31%).

2-Butyroxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (8) Compound **8** was obtained from (±)-*cis*-2-butyryloxy-1-hydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**10**) (method a) or (±)-*cis*-1,2-dibutyryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**11**) (method b) under conditions similar with those described for the preparation of **7**. Purification by flash chromatography (solvent: CH_2Cl_2, then CH_2Cl_2/MeOH 99 : 1 to 95 : 5) gave **5** in 19 and 12% yield, respectively, as orange needles, mp 180 °C (CH_2Cl_2). IR (KBr) cm^{-1}: 3052, 2960, 1756, 1649, 1618, 1587, 1566, 1494, 1460, 1397, 1332, 1211, 1138, 1108, 1084, 1025, 872, 811, 745. UV λ_{max} (MeOH) nm (log ε): 239 (4.43), 275 (4.77), 307 (4.72), 360 (3.95), 440 (3.77). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.90 (1H, s, C8-H), 8.01 (1H, dd, J=9, 1 Hz, C9-H), 7.84 (1H, dd, J=9, 1 Hz, C12-H), 7.65 (1H, s, C13-H), 7.53 (1H, ddd, J=9, 8, 1 Hz, C11-H), 7.40 (1H, ddd, J=9, 8, 1 Hz, C10-H), 6.55 (1H, s, C1-H), 6.32 (1H, s, C5-H), 4.00 (3H, s, O-C$\underline{H}_3$), 3.94 (3H, s, N-C$\underline{H}_3$), 2.52 (2H, t, J=7.5 Hz, C2-COC$\underline{H}_2$CH$_2$CH$_3$), 1.79 (2H, m, C2-COCH$_2$C$\underline{H}_2$CH$_3$), 1.59 (6H, s, C3-(C$\underline{H}_3$)$_2$), 1.07 (3H, t, J=7.5 Hz, C2-COCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 178.0 (C-7), 172.0 (C2-OCO), 163.0 (C-6), 157.6 (C-4a), 150.3 (C-14a), 147.5 (C-2), 141.0 (C-13a), 135.8 (C-12a), 129.6 (C-9), 128.7 (C-8a), 128.2 (C-11+C-8), 126.7 (C-12), 125.7 (C-7a), 124.5 (C-10), 111.9 (C-13), 110.1 (C-6a), 109.7 (C-5), 102.4 (C-14b), 98.8 (C-1), 77.2 (C-3), 56.4 (O$\underline{C}$H$_3$), 44.6 (N$\underline{C}$H$_3$), 36.3 (C2-OCO$\underline{C}$H$_2$CH$_2$CH$_3$), 24.1 (C3($\underline{C}$H$_3$)$_2$), 18.4 (C2-OCOCH$_2$$\underline{C}H_2CH_3$), 13.7 (C2-OCOCH$_2CH_2$$\underline{C}H_3$). EI-MS m/z: 457 $[M]^{+\cdot}$, 387 $[M-CO(CH_2)_3]^{+\cdot}$. *Anal.* Calcd for $C_{28}H_{27}NO_5$: C, 73.51; H, 5.95; N, 3.06. Found: C, 73.58; H, 6.01; N, 2.99.

Pharmacology. Cytotoxicity Murine leukemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD, U.S.A.) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured using the microculture tetrazolium assay essentially as described.[16] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} values (mean, n=3), which are defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

Cell Cycle Analysis For the cell cycle analysis, L1210 cells (5×10^5 cells/ml) were incubated for 21 h with various concentrations of drugs. Cells were then fixed with 70% ethanol (v/v), washed, and incubated in PBS containing RNAse 100 μg/ml and propidium iodide 50 μg/ml for 30 min at 20 °C. For each sample, 10000 cells were analyzed on an XLMCL flow cytometer (Beckman Coulter, France). Results are expressed as the percentage of cells arrested in the given phases of the cell cycle.

Binding to DNA: Gel Shift Studies A typical cross-linking reaction consisted of incubating 8 μl radiolabelled DNA, 2 μl of buffer (10 mM Na cacodylate, pH 7.0; Tris buffer must be avoided due to the presence of reactive amine functions) and 10 μl of the drug at the desired concentration in the dark at room temperature, for 16 h, prior to adding 5 μl of a 50% glycerol solution containing tracking dyes. DNA samples were resolved by electrophoresis under non-denaturing conditions in 6% acrylamide gels for about 5 h at 300 V at room temperature in TBE buffer (89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3). Gels were transferred to Whatman 3MM paper, dried under vacuum at 80 °C, and then analyzed on a phosphorimager (Molecular Dynamics 445SI).

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