# Synthesis and Cytotoxic and Antitumor Activity of 1,2-Dihydroxy-1,2-dihydrobenzo[*b*]acronycine Diacid Hemiesters and Carbamates

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**A series of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diacid hemiesters and dicarbamates were prepared by acylation of *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. The cytotoxicity of the dicarbamates depended on the steric hindrance of the esterifying groups at positions 1 and 2. Diacid hemiesters displayed significant *in vitro* cytotoxic activities and induced cell cycle perturbations similar to those obtained with *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (S23906-1) currently under preclinical development. *cis*-1-Acetoxy-2-hemiglutaryloxy-1,2-dihydrobenzo[*b*]acronycine was the most promizing compound of the series, inducing complete inhibition of tumor growth when tested against C38 colon adenocarcinoma implanted in mice.**

**Key words** acronycine; benzo[*b*]acronycine; antitumor activity

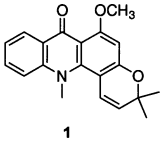
The pyranoacridone alkaloid acronycine (**1**), which was first isolated from *Acronychia baueri* Schott (Rutaceae) in 1948,[1—3] was later shown to exhibit antitumor properties in a panel of murine solid tumor models, including S-180 and AKR sarcomas, X-5563 myeloma, S-115 carcinoma, and S-91 melanoma.[4,5] Nevertheless, its moderate potency and very low solubility in aqueous solvents severely hampered its clinical trials, which have given only poor results.[6] Consequently, the development of structural analogues with increased potency and/or better water solubility was highly desirable.

Our efforts to obtain 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 potency were obtained with derivatives modified in the pyran ring, which had reactivity toward nucleophilic agents similar to that of acronycine epoxide but improved 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, such as diacetate **2**, currently being developed under the code S23906-1, are considered valuable candidates for clinical studies.[10] 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.[11,12]

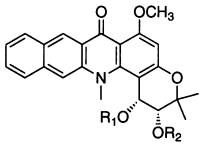
We describe here the synthesis and biological activities of a new series of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]-acronycine diesters, including *bis*-diacid hemiesters, mixed diacid hemiesters, and dicarbamates. These compounds were conceived with the goal of obtaining novel drugs as potent as previously described diesters,[9,12] but with improved solubility in aqueous solvents, which is particularly desirable when a parenteral formulation is envisaged.

**Chemistry** To obtain in a single experiment both (±)-*cis*-1,2-dihemisuccinyloxy-1,2-dihydrobenzo[*b*]acronycine (**3**) and (±)-*cis*-1-acetoxy-2-hemisuccinyloxy-1,2-dihydrobenzo[*b*]acronycine (**4**), the (±)-diol **5**[9] was treated with 1.5 eq of succinic anhydride, in anhydrous pyridine, at room temperature, in the presence of 4-dimethylaminopyridine. After 17 h, a large excess of acetic anhydride was added to the reaction mixture, which was allowed to stand for a further 1.5 h. Column chromatography over silica gel gave the two desired diesters **3** and **4**, in 56% and 20% yield, respectively. The same experimental procedure afforded (±)-*cis*-1,2-dihemiglutaryloxy-1,2-dihydrobenzo[*b*]acronycine (**6**) and (±)-*cis*-1-acetoxy-2-hemiglutaryloxy-1,2-dihydrobenzo[*b*]acronycine (**7**) when glutaric anhydride was used as an acylating agent instead of succinic anhydride.

*bis*-Dialkylcarbamates were obtained when the (±)-*cis*-diol **5** was treated with a slight excess of an appropriate *N*,*N*-dialkylcarbamyl chloride in the presence of potassium hydride in anhydrous tetrahydrofuran (THF). Following this



**1**



(±) **2** $R_1 = R_2 = COCH_3$
(±) **3** $R_1 = R_2 = COCH_2CH_2COOH$
(±) **4** $R_1 = COCH_3$ $R_2 = COCH_2CH_2COOH$
(±) **5** $R_1 = R_2 = H$
(±) **6** $R_1 = R_2 = COCH_2CH_2CH_2COOH$
(±) **7** $R_1 = COCH_3$ $R_2 = COCH_2CH_2CH_2COOH$
(±) **8** $R_1 = R_2 = CON(CH_3)_2$
(±) **9** $R_1 = R_2 = CON(CH_2CH_3)_2$
(±) **10** $R_1 = R_2 = CON(CH(CH_3)_2)_2$
(±) **11** $R_1 = R_2 = CON(CH_2CH_2CH_2CH_3)_2$

Chart 1

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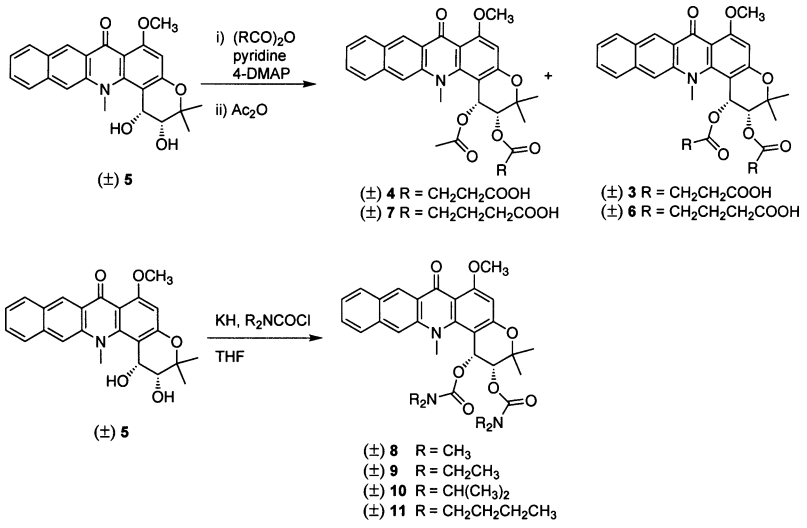


Chart 2

Table 1. Inhibition of L1210 Cell Proliferation and Cell Cycle Perturbation Induced by Compounds **3**, **4** and **6**—**11** in Comparison with (±)-*cis*-1,2-Diacetoxy-1,2-dihydrobenzo[*b*]acronycine (**2**)

| Compound | **2** | **3** | **4** | **6** | **7** | **8** | **9** | **10** | **11** |
|---|---|---|---|---|---|---|---|---|---|
| $IC_{50}$ ($\mu$M) | 0.8 | 1.3 | 2.1 | 1.6 | 0.9 | 0.7 | 1.0 | 8.5 | 6.3 |
| Cell cycle perturbation | 72% G2+M (1 $\mu$M) 73% S (5 $\mu$M) | 57% G2+M (2.5 $\mu$M) 79% S (5 $\mu$M) | 66% G2+M (5 $\mu$M) 69% S (10 $\mu$M) | 67% G2+M (2.5 $\mu$M) 73% S (10 $\mu$M) | 70% G2+M (2.5 $\mu$M) 75% S (10 $\mu$M) | 78% G2+M (2.5 $\mu$M) | 36% G2+M (5 $\mu$M) | Non-specific | Non-specific |

Table 2. Antitumor Activities of Compounds **3**, **4**, **6**, and **7** in Comparison with (±)-*cis*-1,2-Diacetoxy-1,2-dihydrobenzo[*b*]acronycine (**2**) against C38 Colon Adenocarcinoma Implanted in Mice

| Compound | **2** | **3** | **4** | **6** | **7** |
|---|---|---|---|---|---|
| T/C (%) (Tumor growth) | 0 (6.25 mg/kg) | 47 (25 mg/kg) | 46 (12.5 mg/kg) | 11 (25 mg/kg) | 0 (25 mg/kg) |

Results are expressed in percent of tumor growth (T/C) recorded on day 35—40 at the dose giving the best therapeutic effect without toxicity.

procedure, the desired *cis*-1,2-di-(*N*,*N*-dimethyl)carbamyloxy-1,2-dihydrobenzo[*b*]acronycine (**8**) as well as its *N*,*N*-diethyl, *N*,*N*-di-*iso*-propyl, and *N*,*N*-dibutyl counterparts **9**—**11** could be prepared conveniently.

**Pharmacology** The study of the biological properties of the new 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters was carried out *in vitro* in the L1210 murine leukemia cell line. The results ($IC_{50}$ values) are reported in Table 1. The four diacid hemiesters **3**, **4**, **6**, and **7** were markedly cytotoxic, with $IC_{50}$ values within the same range of magnitude as diacetate **2**, currently under preclinical development. The dimethylcarbamate **8** and diethylcarbamate **9** also exhibited significant antiproliferative activity. In contrast, the bulky di-*iso*-propylcarbamate **10** and dibutylcarbamate **11** displayed only marginal activity when compared with the reference compound.

The perturbation of the cell cycle induced by these compounds was studied in the same cell line. Interestingly, the effects of the diacid hemiesters **3**, **4**, **6**, and **7** were very similar to those previously described for diacetate **2**.[13] All these compounds induced a partially reversible accumulation in the $G_2$+M phases of the cell cycle at low concentrations, whereas higher concentrations induced an irreversible arrest in the S phase. The perturbations observed with carbamates **8** and **9** were somewhat different, suggesting that they should act, at the molecular level, through a different mechanism of action. Di-*iso*-propyl and dibutylcarbamates **10** and **11** did not induced any specific cell cycle perturbation.

The four diacid hemiesters, which appeared as the most promising compounds from the above experiments, were further evaluated *in vivo* against the C38 colon adenocarcinoma implanted subcutaneously in mice.[14] Table 2 shows the results in terms of percentage of tumor growth (T/C), at the dose giving the best therapeutic effect without toxicity. Both hemiglutaryl esters **6** and **7** were highly efficient, inhibiting tumor growth by more than 85%.

## Results and Discussion

The effects in terms of cytotoxicity of the esterification of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine by two

carbamyl units depended upon the steric hindrance of the esterifying groups at positions 1 and 2. The best results were obtained with the less bulky *N*,*N*-dimethylcarbamate and *N*,*N*-diethylcarbamate **8** and **9**, respectively.

The four diacid hemiesters **3**, **4**, **6**, and **7** displayed *in vitro* cytotoxic activities comparable with that of the reference compound **2**. Moreover, the typical concentration-dependent perturbations they induced on the cell cycle were similar to those observed with **2**. When tested against C38 adenocarcinoma, hemiglutaryl esters **6** and **7** gave better therapeutic effects than their hemisuccinyl conterparts **3** and **4**. Complete inhibition of the tumor growth was achieved with *cis*-1-acetoxy-2-hemiglutaryloxy-1,2-dihydrobenzo[*b*]acronycine (**7**), which appears to be the most promising derivative of this new series.

## Experimental

**Chemistry** The melting points were determined on a Leica VM apparatus and are not corrected. IR spectra ($\nu_{max}$ in $cm^{-1}$) were obtained on a Perkin-Elmer 257 instrument. UV spectra ($\lambda_{max}$ in nm) were determined in spectroscopic-grade MeOH on a Beckman Model 34 spectrophotometer. $^1$H-NMR ($\delta$ [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 fast atom-bombardment ionization (FAB-MS; matrix: thioglycerol) technique. Flash column chromatographies were performed using silica gel 60 Merck (35—70 $\mu$m) with an overpressure of 300 mbars.

**Preparation of (±)-*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 Diacid Hemiesters. General Method** In a typical experiment, succinic anhydride (148 mg, 1.5 mmol) was added to a solution of (±)-*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 (**5**) (400 mg, 0.98 mmol) in dry pyridine (7 ml) containing 4-dimethylaminopyridine (2 mg). The reaction mixture was stirred at 20 °C for 17 h. After cooling at −15 °C, acetic anhydride (7 ml, 75 mmol) was added and the reaction mixture was allowed to stand at 20 °C for a further 1.5 h and evaporated under reduced pressure. Purification by flash column chromatography (solvent: $CH_2Cl_2$, then $CH_2Cl_2$/MeOH 99 : 1 to 90 : 10) gave successively (±)-*cis*-1,2-dihemisuccinyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**3**) (335 mg, 56%) and (±)-*cis*-1-acetoxy-2-hemisuccinyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**) (105 mg, 20%).

(±)-*cis*-1,2-Dihemisuccinyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**3**): Yellow needles, mp 128 °C ($CH_2Cl_2$). IR (KBr) $cm^{-1}$: 3380, 3050, 2950, 1743, 1641, 1589, 1497, 1400, 1374, 1205, 1152, 1086, 820. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 236 (4.55), 287 (4.99), 338 (4.21), 436 (3.90). $^1$H-NMR (300 MHz, $CDCl_3$/$CD_3OD$ 4 : 1) $\delta$: 1.54 (3H, s, C3–C$\underline{H}_3$), 1.68 (3H, s, C3–C$\underline{H}_3$), 2.42—2.84 (8H, m, 2×OCOC$\underline{H}_2$C$\underline{H}_2$COOH), 3.85 (3H, s, N–C$\underline{H}_3$), 4.05 (3H, s, O–C$\underline{H}_3$), 5.65 (1H, d, $J$=5 Hz, C2–H), 6.44 (1H, s, C5–H), 6.65 (1H, d, $J$=5 Hz, C1–H), 7.48 (1H, td, $J$=8, 1.5 Hz, C10–H), 7.61 (1H, td, $J$=8, 1.5 Hz, C11–H), 8.00 (1H, dd, $J$=8, 1.5 Hz, C12–H), 8.19 (1H, dd, $J$=8, 1.5 Hz, C9–H), 8.13 (1H, s, C13–H), 8.86 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 23.3 (q, C3–$\underline{C}H_3$), 25.5 (q, C3–$\underline{C}H_3$), 29.8 (3C, 3t, 3×$\underline{C}H_2$), 30.8 (t, $\underline{C}H_2$), 43.3 (q, N–$\underline{C}H_3$), 57.1 (q, O–$\underline{C}H_3$), 66.9 (d, C-1), 69.2 (d, C-2), 77.7 (s, C-3), 97.1 (d, C-5), 98.8 (s, C-14b), 111.3 (s, C-6a), 113.8 (d, C-13), 125.6 (d, C-10), 126.5 (s, C-7a), 127.4 (d, C-12), 128.0 (d, C-8), 129.1 (s, C-8a), 129.2 (d, C-11), 130.3 (d, C-9), 136.6 (s, C-12a), 143.0 (s, C-13a), 150.5 (s, C-14a), 160.8 (s, C-4a), 163.2 (s, C-6), 173.1 (s, C1–O–$\underline{C}$O), 173.4 (s, C2–O–$\underline{C}$O), 175.0 (2C, 2s, 2×$\underline{C}$OOH), 177.6 (s, C-7). FAB-MS *m*/*z*: 606 $[MH]^+$. *Anal.* Calcd for $C_{32}H_{31}NO_{11}$: C, 63.47; H, 5.16; N, 2.31. Found: C, 63.54; H, 5.07; N, 2.35.

(±)-*cis*-1-Acetoxy-2-hemisuccinyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**4**): Yellow needles, mp 154 °C ($CH_2Cl_2$/MeOH 4 : 1). IR (KBr) $cm^{-1}$: 3450, 3052, 2979, 1743, 1640, 1588, 1495, 1399, 1374, 1227, 1207, 1151, 1086, 970. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 236 (4.50), 287 (4.97), 339 (4.16), 436 (3.86). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: 1.46 (3H, s, C3–C$\underline{H}_3$), 1.56 (3H, s, C3–C$\underline{H}_3$), 1.97 (3H, s, C1–O–COC$\underline{H}_3$), 2.34—2.88 (4H, m, C2–O–COC$\underline{H}_2$C$\underline{H}_2$COOH), 3.69 (3H, s, N–C$\underline{H}_3$), 4.00 (3H, s, O–C$\underline{H}_3$), 5.50 (1H, d, $J$=5 Hz, C2–H), 6.28 (1H, s, C5–H), 6.55 (1H, d, $J$=5 Hz, C1–H), 7.42 (1H, td, $J$=8, 1.5 Hz, C10–H), 7.55 (2H, m, C11–H, C13–H), 7.84 (1H, dd, $J$=8, 1.5 Hz, C12–H), 8.01 (1H, dd, $J$=8, 1.5 Hz, C9–H), 8.88 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 21.0 (q, C1–O–CO$\underline{C}H_3$), 23.4 (q, C3–$\underline{C}H_3$), 24.4 (q, C3–$\underline{C}H_3$), 28.6 (2C, 2t, CO$\underline{C}H_2\underline{C}H_2$COOH), 43.0 (q, N–$\underline{C}H_3$), 56.1 (q, O–$\underline{C}H_3$), 65.7 (d, C-1), 69.7 (d, C-2), 76.4 (s, C-3), 94.4 (d, C-5), 97.7 (s, C-14b), 111.6 (d, C-13), 112.6 (s, C-6a), 124.5 (d, C-10), 125.6 (s, C-7a), 126.7 (d, C-12), 128.1 (d, C-8), 128.3 (d, C-11), 128.6 (s, C-8a), 129.5 (d, C-9), 135.8 (s, C-12a), 142.2 (s, C-13a), 150.2 (s, C-14a), 160.4 (s, C-4a), 162.9 (s, C-6), 171.6 (s, C1–O–$\underline{C}$O), 172.7 (s, C2–O–$\underline{C}$O), 176.1 (s, $\underline{C}$OOH), 178.4 (s, C-7). FAB-MS *m*/*z*: 548 $[MH]^+$. *Anal.* Calcd for $C_{30}H_{29}NO_9$: C, 65.81; H, 5.34; N, 2.56. Found: C, 65.67; H, 5.34; N, 2.61.

Hemiglutarates **6** and **7** were prepared in 34% and 22% yield, respectively, in a manner similar to that described for **3** and **4** when glutaric anhydride was used instead of succinic anhydride.

(±)-*cis*-1,2-Dihemiglutaryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**6**): Yellow needles, mp 118 °C ($CH_2Cl_2$/$Me_2CO$ 1 : 1). IR (KBr) $cm^{-1}$: 3410, 3051, 2930, 1741, 1646, 1589, 1494, 1399, 1374, 1208, 1150, 1085, 840. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 237 (4.61), 286 (5.09), 338 (4.22), 435 (3.89). $^1$H-NMR (300 MHz, $CDCl_3$/$CD_3OD$ 4 : 1) $\delta$: 1.29 (3H, s, C3–C$\underline{H}_3$), 1.43 (3H, s, C3–C$\underline{H}_3$), 1.54—1.74 (4H, m, 2×OCOCH$_2$C$\underline{H}_2$CH$_2$COOH), 1.90—2.27 (8H, m, 2×OCOC$\underline{H}_2$CH$_2$C$\underline{H}_2$COOH), 3.56 (3H, s, N–C$\underline{H}_3$), 3.84 (3H, s, O–C$\underline{H}_3$), 5.38 (1H, d, $J$=5 Hz, C2–H), 6.17 (1H, s, C5–H), 6.43 (1H, d, $J$=5 Hz, C1–H), 7.25 (1H, td, $J$=8, 1.5 Hz, C10–H), 7.39 (1H, td, $J$=8, 1.5 Hz, C11–H), 7.45 (1H, s, C13–H), 7.73 (1H, dd, $J$=8, 1.5 Hz, C12–H), 7.84 (1H, dd, $J$=8, 1.5 Hz, C9–H), 8.67 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$/$CD_3OD$ 4 : 1) $\delta$: 21.0 (2C, 2t, 2×$\underline{C}H_2$), 23.0 (q, C3–$\underline{C}H_3$), 24.1 (q, C3–$\underline{C}H_3$), 32.7 (2C, 2t, 2×$\underline{C}H_2$), 33.3 (2C, 2t, 2×$\underline{C}H_2$), 42.8 (q, N–$\underline{C}H_3$), 55.7 (q, O–$\underline{C}H_3$), 65.6 (d, C-1), 69.0 (d, C-2), 76.3 (s, C-3), 94.3 (d, C-5), 97.6 (s, C-14b), 110.1 (s, C-6a), 111.7 (d, C-13), 125.5 (d, C-10), 124.9 (s, C-7a), 126.5 (d, C-12), 127.6 (d, C-8), 128.2 (s, C-8a), 128.4 (d, C-11), 129.1 (d, C-9), 135.7 (s, C-12a), 141.8 (s, C-13a), 150.1 (s, C-14a), 160.5 (s, C-4a), 162.6 (s, C-6), 172.9 (s, C1–O–$\underline{C}$O), 173.2 (s, C2–O–$\underline{C}$O), 175.0 (2C, 2s, 2×$\underline{C}$OOH), 178.5 (s, C-7). FAB-MS *m*/*z*: 634 $[MH]^+$. *Anal.* Calcd for $C_{34}H_{35}NO_{11}$: C, 64.45; H, 5.57; N, 2.21. Found: C, 64.51; H, 5.63; N, 2.17.

(±)-*cis*-1-Acetoxy-2-hemiglutaryloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**7**): Yellow needles, mp 155 °C (AcOEt/hexane 4 : 1). IR (KBr) $cm^{-1}$: 3430, 3050, 2978, 1742, 1642, 1589, 1494, 1399, 1228, 1207, 1151, 1086, 1000. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 236 (4.52), 287 (4.96), 337 (4.18), 436 (3.86). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: 1.46 (3H, s, C3–C$\underline{H}_3$), 1.56 (3H, s, C3–C$\underline{H}_3$), 1.82 (2H, m, OCOCH$_2$C$\underline{H}_2$CH$_2$COOH), 1.90 (3H, s, C1–O–COC$\underline{H}_3$), 2.30 (2H, t, $J$=7.5, OCOCH$_2$CH$_2$C$\underline{H}_2$COOH), 2.40 (2H, t, $J$=7.5, OCOC$\underline{H}_2$CH$_2$CH$_2$COOH), 3.70 (3H, s, N–C$\underline{H}_3$), 3.95 (3H, s, O–C$\underline{H}_3$), 5.50 (1H, d, $J$=5 Hz, C2–H), 6.25 (1H, s, C5–H), 6.55 (1H, d, $J$=5 Hz, C1–H), 7.35 (1H, td, $J$=8, 1.5 Hz, C10–H), 7.52 (2H, m, C11–H, C13–H), 7.80 (1H, dd, $J$=8, 1.5 Hz, C12–H), 7.95 (1H, dd, $J$=8, 1.5 Hz, C9–H), 8.86 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 19.6 (t, $\underline{C}H_2$), 21.0 (q, C1–O–CO$\underline{C}H_3$), 23.4 (q, C3–$\underline{C}H_3$), 24.4 (q, C3–$\underline{C}H_3$), 28.2 (2C, 2t, 2×$\underline{C}H_2$), 42.9 (q, N–$\underline{C}H_3$), 56.1 (q, O–$\underline{C}H_3$), 65.8 (d, C-1), 69.4 (d, C-2), 76.3 (s, C-3), 94.3 (d, C-5), 97.6 (s, C-14b), 111.6 (d, C-13), 111.8 (s, C-6a), 124.5 (d, C-10), 125.4 (s, C-7a), 126.6 (d, C-12), 128.1 (d, C-8), 128.3 (d, C-11), 128.6 (s, C-8a), 129.5 (d, C-9), 135.8 (s, C-12a), 142.2 (s, C-13a), 150.2 (s, C-14a), 160.4 (s, C-4a), 162.8 (s, C-6), 171.0 (s, C1–O–$\underline{C}$O), 172.2 (s, C2–O–$\underline{C}$O), 176.5 (s, $\underline{C}$OOH), 178.3 (s, C-7). FAB-MS *m*/*z*: 562 $[MH]^+$. *Anal.* Calcd for $C_{31}H_{31}NO_9$: C, 66.30; H, 5.56; N, 2.49. Found: C, 66.18; H, 5.58; N, 2.45.

**Preparation of (±)-*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 Dicarbamates. General Method** Potassium hydride (80 mg, 0.7 mmol) was added at −10 °C to a solution of (±)-*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 (**5**) (50 mg, 0.12 mmol) in anhydrous THF (4 ml). The apropriate *N*,*N*-dialkylcarbamyl chloride (0.32 mmol) was added dropwise at −10 °C and the mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with ethyl acetate (50 ml) and saturated $NaHCO_3$ aqueous solution (10 ml). The organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (solvent: $CH_2Cl_2$/MeOH 95 : 5) (**8**) or by recrystallization in acetone (**9**—**11**).

(±)-*cis*-1,2-Di-(*N*,*N*-dimethyl)carbamyloxy-6-methoxy-3,3,14-trimethyl-

1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**8**): Yield= 93%, amorphous solid. IR (KBr) $cm^{-1}$: 2932, 1712, 1697, 1646, 1619, 1584, 1499, 1460, 1401, 1355, 1266, 1149, 1106, 1087, 1033, 866, 807, 757. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 251 (4.03), 288 (5.00), 339 (4.17), 437 (3.84). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: 1.49 (3H, s, C3–C$\underline{H}_3$), 1.59 (3H, s, C3–C$\underline{H}_3$), 2.42 (3H, s, CON–C$\underline{H}_3$), 2.61 (3H, s, CON–C$\underline{H}_3$), 2.81 (3H, s, CON–C$\underline{H}_3$), 2.98 (3H, s, CON–C$\underline{H}_3$), 3.74 (3H, s, N14–C$\underline{H}_3$), 4.01 (3H, s, O–C$\underline{H}_3$), 5.41 (1H, d, $J$=5 Hz, C2–H), 6.29 (1H, s, C5–H), 6.42 (1H, d, $J$=5 Hz, C1–H), 7.42 (1H, t, $J$=8 Hz, C10–H), 7.55 (2H, m, C11–H, C13–H), 7.86 (1H, d, $J$=8 Hz, C12–H), 8.03 (1H, d, $J$=8 Hz, C9–H), 8.90 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 23.4 (q, C3–$\underline{C}H_3$), 24.8 (q, C3–$\underline{C}H_3$), 35.3 (q, CON–$\underline{C}H_3$), 35.5 (q, CON–$\underline{C}H_3$), 36.5 (q, CON–$\underline{C}H_3$), 36.7 (q, CON–$\underline{C}H_3$), 44.7 (q, N14–$\underline{C}H_3$), 56.3 (q, O–$\underline{C}H_3$), 67.5 (d, C-1), 70.7 (d, C-2), 77.5 (s, C-3), 94.0 (d, C-5), 98.5 (s, C-14b), 107.9 (s, C-6a), 112.5 (d, C-13), 124.4 (d, C-10), 125.9 (s, C-7a), 126.7 (d, C-12), 128.0 (d, C-8), 128.2 (d, C-11), 128.5 (s, C-8a), 129.6 (d, C-9), 135.9 (s, C-12a), 142.8 (s, C-13a), 150.2 (s, C-14a), 155.5 (s, C-4a), 155.7 (s, C-6), 160.5 (s, C2–O–$\underline{C}O$), 162.7 (s, C1–O–$\underline{C}O$), 177.5 (s, C-7). FAB-MS *m/z*: 458 $[MH]^+$. *Anal.* Calcd for $C_{30}H_{33}N_3O_7$: C, 65.80; H, 6.07; N, 7.67. Found: C, 65.71; H, 6.01; N, 7.71.

(±)-*cis*-1,2-Di-(*N*,*N*-diethyl)carbamyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**9**): Yield= 42%, yellow needles, mp 193 °C. IR (KBr) $cm^{-1}$: 2974, 2928, 1704, 1693, 1642, 1619, 1584, 1495, 1479, 1460, 1429, 1398, 1281, 1149, 1084, 757. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 251 (4.05), 288 (4.99), 340 (4.19), 437 (3.87). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: 0.52 (3H, t, $J$=7 Hz, $CONCH_2C\underline{H}_3$), 0.79 (3H, t, $J$=7 Hz, $CONCH_2C\underline{H}_3$), 1.03 (3H, t, $J$=7 Hz, $CONCH_2C\underline{H}_3$), 1.20 (3H, t, $J$=7 Hz, $CONCH_2C\underline{H}_3$), 1.48 (3H, s, C3–C$\underline{H}_3$), 1.60 (3H, s, C3–C$\underline{H}_3$), 2.70—3.40 (8H, m, 4×$CONC\underline{H}_2CH_3$), 3.76 (3H, s, N–C$\underline{H}_3$), 4.00 (3H, s, O–C$\underline{H}_3$), 5.49 (1H, d, $J$=5 Hz, C2–H), 6.28 (1H, s, C5–H), 6.47 (1H, d, $J$=5 Hz, C1–H), 7.41 (1H, t, $J$=8 Hz, C10–H), 7.53 (2H, m, C11–H, C13–H), 7.82 (1H, d, $J$=8 Hz, C12–H), 8.02 (1H, d, $J$=8 Hz, C9–H), 8.90 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 13.3 (3C, 3q, 3×$CONCH_2\underline{C}H_3$), 13.6 (q, $CONCH_2\underline{C}H_3$), 22.9 (q, C3–$\underline{C}H_3$), 24.9 (q, C3–$\underline{C}H_3$), 40.8 (t, $CON\underline{C}H_2CH_3$), 41.5 (t, $CON\underline{C}H_2CH_3$), 41.9 (t, $CON\underline{C}H_2CH_3$), 42.0 (t, $CON\underline{C}H_2CH_3$), 42.7 (q, N–$\underline{C}H_3$), 56.3 (q, O–$\underline{C}H_3$), 67.2 (d, C-1), 70.0 (d, C-2), 76.4 (s, C-3), 94.0 (d, C-5), 98.8 (s, C-14b), 107.9 (s, C-6a), 111.8 (d, C-13), 124.4 (d, C-10), 125.5 (s, C-7a), 126.6 (d, C-12), 128.0 (d, C-8), 128.2 (d, C-11), 128.6 (s, C-8a), 129.6 (d, C-9), 135.7 (s, C-12a), 142.1 (s, C-13a), 150.2 (s, C-14a), 154.7 (s, C-4a), 154.9 (s, C-6), 160.7 (s, C2–O–$\underline{C}O$), 162.8 (s, C1–O–$\underline{C}O$), 178.2 (s, C-7). FAB-MS *m/z*: 604 $[MH]^+$. *Anal.* Calcd for $C_{34}H_{41}N_3O_7$: C, 67.64; H, 6.85; N, 6.96. Found: C, 67.70; H, 6.82; N, 7.03.

(±)-*cis*-1,2-Di-(*N*,*N*-di-iso-propyl)carbamyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**10**): Yield=36%, yellow needles, mp 150 °C. IR (KBr) $cm^{-1}$: 2966, 2935, 1693, 1646, 1619, 1592, 1568, 1487, 1440, 1401, 1297, 1207, 1153, 1083, 761. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 251 (4.03), 289 (4.97), 340 (4.16), 437 (3.87). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: 0.61—0.70 (9H, m, 3×C$\underline{H}_3$), 1.00 (3H, d, $J$=7 Hz, C$\underline{H}_3$), 1.06 (3H, d, $J$=7 Hz, C$\underline{H}_3$), 1.15 (3H, d, $J$=7 Hz, C$\underline{H}_3$), 1.30 (3H, d, $J$=7 Hz, C$\underline{H}_3$), 1.39 (3H, d, $J$=7 Hz, C$\underline{H}_3$), 1.43 (3H, s, C3–C$\underline{H}_3$), 1.59 (3H, s, C3–C$\underline{H}_3$), 3.33 (1H, hept, $J$=7 Hz, $C\underline{H}(CH_3)_2$), 3.59 (2H, m, 2×$C\underline{H}(CH_3)_2$), 3.74 (3H, s, N–C$\underline{H}_3$), 3.97 (3H, s, O–C$\underline{H}_3$), 4.16 (1H, hept, $J$=7 Hz, $C\underline{H}(CH_3)_2$), 5.62 (1H, d, $J$=5 Hz, C2–H), 6.25 (1H, s, C5–H), 6.51 (1H, d, $J$=5 Hz, C1–H), 7.37 (1H, t, $J$=8 Hz, C10–H), 7.49 (2H, m, C11–H, C13–H), 7.77 (1H, d, $J$=8 Hz, C12–H), 7.98 (1H, d, $J$=8 Hz, C9–H), 8.88 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 20.0 (q, $\underline{C}H_3$), 20.5 (q, $\underline{C}H_3$), 20.6 (q, $\underline{C}H_3$), 20.7 (q, $\underline{C}H_3$), 20.8 (2q, 2×$\underline{C}H_3$), 21.1 (q, $\underline{C}H_3$), 21.9 (q, $\underline{C}H_3$), 23.1 (q, C3–$\underline{C}H_3$), 24.9 (q, C3–$\underline{C}H_3$), 42.6 (q, N–$\underline{C}H_3$), 44.7 (d, $\underline{C}H(CH_3)_2$), 45.6 (d, $\underline{C}H(CH_3)_2$), 46.4 (d, $\underline{C}H(CH_3)_2$), 47.1 (d, $\underline{C}H(CH_3)_2$), 56.4 (q, O–$\underline{C}H_3$), 66.7 (d, C-1), 69.8 (d, C-2), 77.6 (s, C-3), 94.2 (d, C-5), 99.2 (s, C-14b), 110.0 (s, C-6a), 111.9 (d, C-13), 124.4 (d, C-10), 125.3 (s, C-7a), 126.6 (d, C-12), 128.1 (d, C-8), 128.2 (d, C-11), 128.6 (s, C-8a), 129.6 (d, C-9), 135.7 (s, C-12a), 141.9 (s, C-13a), 150.0 (s, C-14a), 153.7 (s, C-4a), 154.8 (s, C-6), 160.7 (s, C2–O–$\underline{C}O$), 162.7 (s, C1–O–$\underline{C}O$), 176.0 (s, C-7). FAB-MS *m/z*: 660 $[MH]^+$. *Anal.* Calcd for $C_{38}H_{49}N_3O_7$: C, 69.17; H, 7.49; N, 6.37. Found: C, 69.12; H, 7.57; N, 6.32.

(±)-*cis*-1,2-Di-(*N*,*N*-dibutyl)carbamyloxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**11**): Yield= 31%, yellow needles, mp 138 °C. IR (KBr) $cm^{-1}$: 2955, 2928, 1697, 1650, 1619, 1588, 1487, 1405, 1153, 1087, 757. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 251 (4.06), 289 (5.00), 339 (4.18), 437 (3.88). $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$: −0.10 (3H, t, $J$=7 Hz, C$\underline{H}_3$), 0.38—0.54 (2H, m, C$\underline{H}_2$), 0.66 (3H, t, $J$=7 Hz, C$\underline{H}_3$), 0.87 (3H, t, $J$=7 Hz, C$\underline{H}_3$), 0.89—0.98 (4H, m, 2×C$\underline{H}_2$), 1.00 (3H, t, $J$=7 Hz, C$\underline{H}_3$), 1.07—1.29 (4H, m, 2×C$\underline{H}_2$), 1.40 (4H, m, 2×C$\underline{H}_2$), 1.48 (3H, s, C3–C$\underline{H}_3$), 1.52—1.62 (5H, m, C3–C$\underline{H}_3$, C$\underline{H}_2$), 2.50—3.37 (8H, m, 4×$CONC\underline{H}_2$), 3.77 (3H, s, N–C$\underline{H}_3$), 3.99 (3H, s, O–C$\underline{H}_3$), 5.47 (1H, d, $J$=5 Hz, C2–H), 6.26 (1H, s, C5–H), 6.46 (1H, d, $J$=5 Hz, C1–H), 7.38 (1H, t, $J$=8 Hz, C10–H), 7.48 (2H, m, C11–H, C13–H), 7.82 (1H, d, $J$=8 Hz, C12–H), 8.00 (1H, d, $J$=8 Hz, C9–H), 8.89 (1H, s, C8–H). $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$: 12.6 (q, $\underline{C}H_3$), 13.7 (q, $\underline{C}H_3$), 13.8 (q, $\underline{C}H_3$), 13.9 (q, $\underline{C}H_3$), 19.5 (t, $\underline{C}H_2$), 19.9 (t, $\underline{C}H_2$), 20.1 (2C, 2t, 2×$\underline{C}H_2$), 22.6 (q, C3–$\underline{C}H_3$), 24.9 (q, C3–$\underline{C}H_3$), 30.1 (t, $\underline{C}H_2$), 30.2 (t, $\underline{C}H_2$), 30.4 (t, $\underline{C}H_2$), 30.8 (t, $\underline{C}H_2$), 42.5 (q, N–$\underline{C}H_3$), 46.7 (t, CON–$\underline{C}H_2$), 47.2 (t, CON–$\underline{C}H_2$), 47.6 (2C, 2t, 2×CON–$\underline{C}H_2$), 56.1 (q, O–$\underline{C}H_3$), 67.3 (d, C-1), 69.7 (d, C-2), 77.6 (s, C-3), 93.7 (d, C-5), 98.3 (s, C-14b), 110.3 (s, C-6a), 111.9 (d, C-13), 124.3 (d, C-10), 125.3 (s, C-7a), 126.5 (d, C-12), 127.9 (d, C-8), 128.1 (d, C-11), 128.6 (s, C-8a), 129.6 (d, C-9), 135.7 (s, C-12a), 141.9 (s, C-13a), 149.9 (s, C-14a), 154.9 (s, C-4a), 155.2 (s, C-6), 160.5 (s, C2–O–$\underline{C}O$), 162.6 (s, C1–O–$\underline{C}O$), 177.5 (s, C-7). FAB-MS *m/z*: 716 $[MH]^+$. *Anal.* Calcd for $C_{42}H_{57}N_3O_7$: C, 70.46; H, 8.03; N, 5.87. Found: C, 70.32; H, 8.09; N, 5.85.

**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 $\mu$g/ml and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured using the microculture tetrazolium assay essentially as described.[14] 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 ($5\times10^5$ 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 $\mu$g/ml and propidium iodide 50 $\mu$g/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.

**Antitumor Activity** The antitumor activity of the compounds was evaluated in the murine colon 38 adenocarcinoma implanted in B6D2F1 (C57B1/6×DBA2) mice. The colon adenocarcinoma C38 (National Cancer Institute, Frederick, MD, U.S.A.) was introduced by subcutaneous implantation of a tumor fragment into the dorsal flank. The drugs were solubilized in 10% solutol HS15 and administered by intravenous injection on days 10 and 20 after tumor implantation. The tumor volume was measured twice weekly and the results are expressed as percent T/C (median tumor volume in treated animals divided by median tumor volume of controls)×100. Results are expressed at the lowest T/C (highest antitumor activity) obtained at the highest non-toxic dose, recorded on days 35—40. A dose is considered toxic when the weight loss is higher than 20% or when it induces toxic death.

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