Synthesis and Cytotoxic and Antitumor Activity of 1,2-Dihydroxy-1,2-dihydrobenzo[\(b\)]acronycine Diacid Hemiesters and Carbamates

Huong Doan Thi Mai, a Thomas Gaslonde, a Sylvie Michel, a Michel Koch, a François Tillequin, *, a Bruno Pfieffer, b Pierre Renard, b Laurence Kraus-Berthier, c Stéphane Léonce, c and Alain Pierré c

a Laboratoire de Pharmacognosie de l’Université René Descartes, U.M.R./C.N.R.S. n° 8638, Faculté des Sciences Pharmaceutiques et Biologiques; 4, Avenue de l’Observatoire, F-75006 Paris, France; b Les Laboratoires Servier; 1 rue Carle Hébert, 92415 Courbevoie Cedex, France; and c Institut de Recherches Servier, Division Recherche Cancérologie; Pharmaceutiques et Biologiques; 4, Avenue de l’Observatoire, F-75006 Paris, France: and c Institut de Recherches Servier, Division Recherche Cancérologie;

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-diacetoxo-1,2-dihydrobenzo[\(b\)]acronycine (S23906-1) currently under preclinical development. cis-1-Acetoxy-2-hemilgutaryloxy-1,2-dihydrobenzo[\(b\)]acronycine was the most promising compound of the series, inducing complete inhibition of tumor growth when tested against C38 colon adenocarcinoma implanted in mice.

Key words acronycine; benzob[\(b\)]acronycine; antitumor activity

The pyranooacidrnone 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-dihydroacronycine8 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 (\(\pm\))-cis-1,2-dihemisuccinylxoxy-1,2-dihydrobenzo[\(b\)]acronycine (3) and (\(\pm\))-cis-1-acetoxy-2-hemissuccinylxoxy-1,2-dihydrobenzo[\(b\)]acronycine (4), the (\(\pm\))-diol 5 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 (\(\pm\))-cis-1,2-dihemilgutaryloxy-1,2-dihydrobenzo[\(b\)]acronycine (6) and (\(\pm\))-cis-1-acetoxy-2-hemilgutaryloxy-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 (\(\pm\))-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

* To whom correspondence should be addressed. e-mail: francois.tillequin@univ-paris5.fr © 2004 Pharmaceutical Society of Japan
procedure, the desired \textit{cis}-1,2-di-(N,N-dimethyl)carbamyl-oxy-1,2-dihydrobenzo[b]acronycine (8) as well as its N,N-di-ethyl, N,N-di-\textit{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 \textit{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.\textsuperscript{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-\textit{iso}-propyl and dibutylcarbamates 10 and 11 did not induce 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.\textsuperscript{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.

**Results and Discussion** The effects in terms of cytotoxicity of the esterification of \textit{cis}-1,2-dihydroxy-1,2-dihydrobenzo[b]acronycine by two

---

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ ($\mu$m)</td>
<td>0.8</td>
<td>1.3</td>
<td>2.1</td>
<td>1.6</td>
<td>0.9</td>
<td>0.7</td>
<td>1.0</td>
<td>8.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Cell cycle perturbation</td>
<td>72% G2+M</td>
<td>57% G2+M</td>
<td>66% G2+M</td>
<td>67% G2+M</td>
<td>70% G2+M</td>
<td>78% G2+M</td>
<td>78% G2+M</td>
<td>36% G2+M</td>
<td></td>
</tr>
<tr>
<td>(1 $\mu$m)</td>
<td>(2.5 $\mu$m)</td>
<td>(5 $\mu$m)</td>
<td>(2.5 $\mu$m)</td>
<td>(2.5 $\mu$m)</td>
<td>(2.5 $\mu$m)</td>
<td>(5 $\mu$m)</td>
<td>Non-specific</td>
<td>Non-specific</td>
<td></td>
</tr>
<tr>
<td>(5 $\mu$m)</td>
<td>73% S</td>
<td>79% S</td>
<td>69% S</td>
<td>73% S</td>
<td>75% S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/C (%)</td>
<td>0 (6.25 mg/kg)</td>
<td>47 (25 mg/kg)</td>
<td>46 (12.5 mg/kg)</td>
<td>11 (25 mg/kg)</td>
<td>0 (25 mg/kg)</td>
</tr>
</tbody>
</table>

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.
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-dimethylamide and N,N-diethylamide 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 hemisuccinic conterparts 3 and 4. Complete inhibition of the tumor growth was achieved with cis-1-acetoxy-2-hemiglutaric acid (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 (νmax in cm⁻¹) were obtained on a Perkin-Elmer 521 instrument. UV spectra (λmax in nm) were determined in spectrograde-μECD on a Beckman Model 34 spectrophotometer. ¹H-NMR (δ [ppm], J [Hz]) and ¹³C-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: ¹H–¹H COSY, ¹H–¹H NOESY, ¹³C–¹H HMBC, and ¹³C–¹³C HMBC. These experiments were performed using standard Bruker microprocedures. Mass spectra were recorded with a Nermag R-10-10C spectrometer using fast atom bombardment ionization (FAB-MS; matrix: thioglycolate) technique. Flash column chromatographies were performed using silica gel 60 Merck (35–70 μm) with an overpressure of 300 mbar.

**Preparation of cis-1,2-Dihydroxymethoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzof[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, 1205, 1150, 1086, 1000. UV λmax (MeOH) nm (log ε) : 236 (4.55), 287 (4.99), 338 (4.21), 436 (3.90). ¹H-NMR (300 MHz, CDCl₃, CDCl₃/CD₃OD 4:1) δ: 1.54 (3H, c–H₃), 1.68 (3H, c–H₃), 2.42–2.84 (8H, m, 2OCCOCH₂COOH), 3.85 (3H, s, N–CH₃), 4.05 (3H, s, O–CH₃), 5.65 (1H, d, J=5Hz, C2-H), 6.44 (1H, s, C3–H), 6.65 (1H, d, J=5Hz, C5–H), 7.48 (1H, td, J=8, 1.5Hz, C10–H), 7.61 (1H, td, J=8, 1.5Hz, C11–H), 8.00 (1H, d, J=8, 1.5Hz, C12–H), 8.19 (1H, dd, J=8, 1.5Hz, C8–H), 8.13 (1H, c–H₃), 8.86 (1H, s, C–H₈). ¹³C-NMR (75 MHz, DMDSO-d₆) δ: 23.3 (3c, C–3H₃), 25.5 (3c, C–3H₃), 29.8 (3c, C–3H₃), 30.8 (3c, C–3H₃), 30.8 (3c, C–3H₃), 43.3 (q, C–N–CH₃), 57.1 (q, C–CH₃), 66.9 (d, C–1), 69.2 (d, C–2), 77.7 (s, C–3), 97.1 (s, C–5), 98.8 (s, C–14b), 113.8 (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), 168.0 (s, C–4a), 163.2 (s, C–6), 173.1 (s, C–1O–CO), 173.4 (s, C–2O–CO), 175.0 (2c, 2OCCOCH₂COOH), 177.6 (s, C–7). FAB-MS m/z: 626 [MH⁺].

**Preparation of cis-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzof[b]pyrano[3,2-h]acridin-7-one (6)**

Yellow needles, mp 155°C (AcOEt/hexane 4:1). IR (KBr) cm⁻¹: 3430, 3050, 2978, 1742, 1642, 1589, 1494, 1399, 1228, 1205, 1150, 1086, 1000. UV λmax (MeOH) nm (log ε) : 236 (4.55), 287 (4.96), 337 (4.18), 436 (3.86). ¹H-NMR (300 MHz, CDCl₃, CDCl₃/CD₃OD 4:1) δ: 1.54 (3H, c–H₃), 1.68 (3H, c–H₃), 2.42–2.84 (8H, m, 2OCCOCH₂COOH), 3.85 (3H, s, N–CH₃), 4.05 (3H, s, O–CH₃), 5.65 (1H, d, J=5Hz, C2-H), 6.44 (1H, s, C3–H), 6.65 (1H, d, J=5Hz, C5–H), 7.48 (1H, td, J=8, 1.5Hz, C10–H), 7.61 (1H, td, J=8, 1.5Hz, C11–H), 8.00 (1H, d, J=8, 1.5Hz, C12–H), 8.19 (1H, dd, J=8, 1.5Hz, C8–H), 8.13 (1H, c–H₃), 8.86 (1H, s, C–H₈). ¹³C-NMR (75 MHz, CDCl₃) δ: 23.3 (3c, C–3H₃), 25.5 (3c, C–3H₃), 29.8 (3c, C–3H₃), 30.8 (3c, C–3H₃), 43.3 (q, C–N–CH₃), 57.1 (q, C–CH₃), 66.9 (d, C–1), 69.2 (d, C–2), 77.7 (s, C–3), 97.1 (s, C–5), 98.8 (s, C–14b), 113.8 (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), 168.0 (s, C–4a), 163.2 (s, C–6), 173.1 (s, C–1O–CO), 173.4 (s, C–2O–CO), 175.0 (2c, 2OCCOCH₂COOH), 177.6 (s, C–7). FAB-MS m/z: 626 [MH⁺].

**Preparation of cis-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzof[b]pyrano[3,2-h]acridin-7-one (5)**

(50 mg, 0.12 mmol) in anhydrous THF (4 ml). The appropriate N,N-diakylcarbamyl chloride (0.32 mmol) was added dropwise at –10°C and the mixture was stirred at room temperature. After 3h, the reaction mixture was diluted with ethyl acetate (50ml) and saturated NaHCO₃ aqueous solution (10ml). The organic layer was washed with water, dried over anhydrous MgSO₄, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (solvent: CH₂Cl₂/MeOH 95:5) or by recrystallization in acetonitrile (9:1).
(3H, s, C3–CH3), 1.52—1.62 (8H, m, 3, 4-CONH2), 2.50—3.37 (8H, m, 4-CONH2), 3.77 (3H, s, N–CH3), 3.99 (3H, s, O–CONH2), 5.17 (4H, 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). 13C-NMR (75 MHz, CDCl3) δ: 12.9 (6C, 6t, 6CH3), 13.8 (7C, 7CH3), 13.9 (9CH3), 19.5 (t, CH3), 19.9 (t, CH3), 20.1 (2C, 2t, 2CH3), 21.6 (2C, 2CONH), 24.9 (2C, 2CH3), 25.5 (2C, 2CH3), 26.1 (CH3), 30.6 (CH3), 32.3 (C–CH3), 34.2 (C–CH3), 36.7 (q, CON–C–CH3), 41.9 (t, CH(CH3)2), 42.7 (q, N–C–CH3), 43.7 (s, CH–O–C–), 44.7 (q, N14–C–CH3), 43.7 (h), 50.9 (C–CONH2), 56.0 (2C, 2CH3), 59.5 (2C, 2CH3), 62.8 (C–CH3), 63.3 (C–CH3), 65.2 (C–CH3), 75.7 (C5–CH3), 77.5 (C1–CH3), 79.7 (C–CH3), 80.8 (t, C–CH3), 82.5 (C–CH3), 84.7 (t, CON–C–CH3), 84.7 (t, CON–C–CH3), 90.6 (CF3), 126.0 (CH3), 126.7 (C–CH3), 126.8 (C–CH3), 127.0 (C–CH3), 127.0 (C–CH3), 127.8 (C–CH3), 128.0 (C–CH3), 128.6 (C–CH3), 129.4 (C–CH3), 131.7 (C–CH3), 134.7 (C–CH3), 135.7 (C–CH3), 136.0 (C–CH3), 140.9 (C–CH3), 141.4 (C–CH3), 147.1 (C–CH3), 150.0 (C–CH3), 151.6 (C–CH3), 152.6 (C–CH3), 153.7 (C–CH3), 154.0 (C–CH3), 154.5 (C–CH3), 155.0 (C–CH3), 155.0 (C–CH3), 155.4 (C–CH3), 155.5 (C–CH3), 155.6 (C–CH3), 161.5 (C–CH3), 162.6 (C–CH3), 170.0 (C–CH3), 171.5 (C–CH3), 175.5 (C–CH3), 177.5 (C–CH3), 179.7 (C–CH3). FAB-MS m/z: 458 [MH]+. Anal. Caled for C19H25NO7: C 67.0; H 6.82; N 7.03. 8) Hughes G. K., Lahey F. N., Price J. R., Nature (London), 162, 225—244 (1948). 9) Macdonald P. L., Robertson, A. V., Aust. J. Chem., 25, 197—281 (1966). 10) Tillequin F., Michel S., Skalskouis A.-L., “Alkaloids: Chemical and Biological Perspectives,” Vol. 12, ed. by Pefierre S. W., Elsevier, New York, 1998, pp. 1—102. 11) Svoboda G. H., Lloydia, 29, 206—224 (1966). 12) Svoboda G. H., Poore P. G., Simpson P. J., Boder G. B., J. Pharm. Pharmacol., 55, 758—768 (1966). 13) Scarffe J. H., Beaumont A. R., Gowther D., Cancer Treat. Rep., 67, 93—94 (1983). 14) Brum-Bousquet M., Mitaku S., Skalskouis A.-L., Tillequin F., Koch M., Planta Med., 54, 470—471 (1988). 15) Elomri A., Mitaku S., Michel S., Skalskouis A.-L., Tillequin F., Koch M., Pfeiffer B., Guibaud N., Leonse, S., Kraus-Berthier L., Rolland Y., Atassi G., J. Med. Chem., 39, 4762—4766 (1996). 16) Conforti C., Leonse, S., Pfeiffer B., Guibaud N., Leonse, S., Kraus-Berthier L., Rolland Y., Atassi G., J. Med. Chem., 39, 2395—2402 (2000). 17) David-Cordonnier M.-H., Laine W., Lansiaux A., Kouach M., Briand Bongui J.-B., Elomri A., Seguin E., Pfeiffer B., Renard P., David-Cor-
