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-acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-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-7H-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-acetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]pyrano[3,2-h]acridin-7-ones (9, 10) or (±)-cis-1,2-diacetoxy-6-methoxy-3,3,14-trimethyl-1,2,3,14-tetrahydro-7H-benzo[b]pyrano[3,2-h]acridin-7-ones (2, 11) with hydrochloric acid gave the corresponding 2-acetoxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-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-7H-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) has shown antitumor properties in a panel of murine solid tumor models, including S-180 and AKR sarcomas, X-563 myeloma, S-115 carcinoma, and S-91 melanoma. However, it is characterized by the absence of a 1,2-double bond in the benzo[b]pyrano[3,2-h]acridin-7-ones.9) Consequently, the development of compounds with increased potency and/or better water solubility was highly desirable.

Our efforts toward the obtainment of more potent derivatives were performed by a hypothesis of bioactivation of the 1,2-double bond of acronycine into the corresponding epoxide in vivo. Significant improvements in terms of solubility and potency were obtained with derivatives modified in the pyrano 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 and diesters of cis-1,2-dihydroxy-1,2-dihydroacronycine. Representatives of this latter series are considered as valuable drug candidates for clinical studies. 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.

In the course of the exploration of the structure activity relationships in the acronycine series, 2-nitroacronycine, although shown to be toxic in vivo, demonstrated remarkably high potency when tested against a battery of cultured mammalian cells in vitro. 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 nucleophilic targets. Additionally, enol esters position 2 were also worth exploring for comparison purposes. We describe here the synthesis and biological activities of various 2-acetyl- and 2-acetoxy-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-ones.

Chemistry

Treatment of 6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-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-acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-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-7H-benzo[b]pyrano[3,2-h]acridin-7-one (4), 2-butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (5), and 2-benzoyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (6) were prepared in 96, 54, and 42% yield, respectively.
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-acetoxy-6-methoxy-3,14-trimethyl-1,2,3,14-tetrahydro-7H-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-7H-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-acetylbenzo[b]acronyinces 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 (IC₅₀ 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 IC₅₀ 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 7 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.

### Results and Discussion

Considering the structure activity relationships in the benzo[b]acronyince 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]acronyince chromophore.

Enol esters at position 2, exemplified by 7 and 8, displayed cytotoxic activities within the same range of magnitude as (±)-cis-1,2-diacyloxy-1,2-dihydro benzo[b]acronyince. 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 (νₒₒₑₑₑₑ in cm⁻¹) were obtained on a Perkin-Elmer 257 instrument. UV spectra (λₒₒₒₒₒₒₒₒ in nm) were determined in spectroscopic-grade MeOH 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 HMQC, and ¹³C–¹H 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 over-pressure of 300 mbar.

**2-Acetyl-6-methoxy-3,14-trimethyl-3,14-dihydro-7H-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-3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one (3) (50 mg, 0.1374 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (3×300 ml). The combined organic layer was washed with saturated Na₂CO₃ aqueous solution (100 ml), water (2×100 ml), dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. Flash chromatography (solvent: CH₂Cl₂ then CH₂Cl₂/MeOH 99: 1 to 95: 5) gave 4 (54 mg, 96%) as yellow needles, mp

<table>
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<th>Compound</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (µM)</td>
<td>0.8</td>
<td>15</td>
<td>20</td>
<td>30</td>
<td>&gt;50</td>
<td>0.75</td>
<td>1.8</td>
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**Table 1. Inhibition of L1210 Cell Proliferation by Compounds 4—8 in Comparison with Benzo[b]acronyince (3) and S23906-1 (2)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>2</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cycle perturbation</td>
<td>72% G2+M (1 µM)</td>
<td>55% G1 (20 µM)</td>
<td>51% G2+M (20 µM)</td>
</tr>
<tr>
<td>In vitro DNA Alkylation</td>
<td>++</td>
<td>0</td>
<td>0</td>
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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.
27°C (EtOH). IR (KBr) cm⁻¹: 3050, 2973, 2940, 1647, 1602, 1549, 1545, 1406, 1328, 1209, 1123, 1087, 809, 735. UV λₘₐₓ (MeOH) nm (log ε): 236 (4.03), 271 (4.43), 349 (4.56), 404 (3.85). ¹³NMR (400 MHz, CDCl₃): δ: 8.94 (1H, s, CH₃), 8.05 (1H, dd, J = 1, 1 H, C9-H), 7.89 (1H, dd, J = 9, 1, H, C12-H), 7.73 (1H, s, CH₃), 7.59 (1H, dd, J = 9, 8, 1H, C11-H), 7.55 (1H, s, C1-H), 7.47 (1H, dd, J = 9, 8, 1H, C10-H), 6.29 (1H, s, C5-H), 4.03 (1H, s, O-CH₃), 3.99 (1H, s, N-CH₃), 2.47 (8H, s, C₂-COCH₃). 1.72 (6H, s, CH₃-C₃). ¹³C-NMR (75 MHz, CDCl₃): δ: 195.2 (C₂-O), 177.5 (C-7), 165.9 (C-6), 161.3 (14a), 148.4 (14a), 142.1 (C-1), 135.3 (C₂), 132.6 (C₂), 131.5 (C₁), 129.6 (C-9), 129.0 (C₈), 128.5 (C-11), 128.3 (C₁₂), 128.6 (C₁₂), 125.4 (C₇a), 124.9 (14a), 124.9 (14a), 121.0 (C₁₃), 109.6 (C-6a), 104.2 (14b), 93.7 (C-5), 79.5 (C-3), 56.6 (OCH₃), 45.4 (NC=CH₂), 26.2 (C₂-COCH₃), 25.9 (C₃-Cₚ). El-MS m/z: 413 [M⁺] +, 398 [M⁻CH₃]⁺. Anal. Calc. for C₂₈H₂₇NO₅: 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-1,2,3,14-dihydro-7H-benzo[pyrazolo][3,2-b]acridin-7-one (5)** Compound 5 was prepared from 3-benzyl benzaldehyde (34 mg, 0.13 mmol) and 2-butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[pyrazolo][3,2-b]acridin-7-one (2) (40 mg, 0.08 mmol) afforded 7 (11 mg, 31%).

**References**


