Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives

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The usefulness of sodium artesunate (3), a water-soluble derivative of artemisinin (1), is impaired by its poor stability in aqueous solution. To overcome the ease of hydrolysis of the ester group in 3, a new series of derivatives of dihydroartemisinin (2) was prepared in which the solubilizing moiety, which contains a carboxylate group, is joined to dihydroartemisinin by an ether rather than an ester linkage. The new derivatives were prepared in good yield by treatment of dihydroartemisinin with an appropriate alcohol under boron trifluoride etherate catalysis at room temperature. All major condensation products are the 3 isomer. Hydrolysis of the esters with 2.5% KOH-MeOH gave the corresponding potassium salts, which were converted to free acids (8b-d) by acidification. The derivatives were tested in vitro against two clones of human malaria, Plasmodium falciparum D-6 (Sierra Leone clone) and W-2 (Indochina clone). No cross-resistance to the antimalarial agents mefloquine, chloroquine, pyrimethamine, sulfadoxine, and quinine was observed. In general, the new compounds are more effective against the W-2 than the D-6 strain. Esters (5a-d) possess activity comparable to that of the parent compounds 1 and 2; however, conversion of the esters to their corresponding carboxylic acids (7a-d) or acids (8b-d), with the exception of artelanic acid (8d), drastically decreases the antimalarial activities in both cell lines. Artesinic acid, which is both soluble and stable in 2.5% KCl solution, possesses superior in vivo activity against Plasmodium berghei than artemisinin or artesunic acid.

Artemisinin (qinghaosu, 1), a clinically useful antimalarial agent that was isolated from the plant Artemisia annua, is an unusual sesquiterpene lactone containing an epoxide function. Dihydroartemisinin (2), obtained by sodium borohydride reduction of 1, was reported1 to be more therapeutically active than the parent compound.

Scheme 1

Neither 1 nor 2 exhibit cross-resistance to chloroquine and both were proven efficacious against cerebral malaria in humans. Sodium artesunate (3f), the salt of the succinic acid half-ester derivative of dihydroartemisinin, is water soluble and can be administered by intravenous injection. This makes the compound particularly useful in the
treatment of cerebral malaria where rapid reversal of the parasitemia is the goal. The utility of sodium artesunate is, however, impaired by its poor stability in aqueous solution due to the ease of hydrolysis of the ester linkage.

In order to overcome the stability problem, we have prepared a series of new water-soluble, stable derivatives in which the solubilizing group, carboxylate, is on a moiety that is joined to dihydroartemisinin by an ether rather than an ester linkage.

Chemistry

Dihydroartemisinin (2) was prepared by sodium borohydride reduction of 1 according to a modified literature procedure. The peroxide group is not affected by the sodium borohydride treatment. Inasmuch as dihydroartemisinin is a hemiacetal (lactol), it exists as a mixture of α and β anomers whose ratio is solvent dependent. The new ether derivatives of dihydroartemisinin (5a-d) were prepared by treatment of 2 with an appropriate alcohol (4) under the catalysis of boron trifluoride etherate. The compound was monitored by 1H NMR. In a similar manner, the reactions. The methanol or ethanol needed for the formation of 6a or 6b is very likely generated by trans-esterification or lactone formation in 2.5% NaHCO₃ solution at room temperature and monitored by 1H NMR.

Results and Discussion

The new water-soluble dihydroartemisinin derivatives were tested in vitro against two clones of human malaria, Plasmodium falciparum D-6 (Sierra Leone clone) and W-2 (Indochina clone). The former clone is a strain that is resistant to mefloquine and the latter to chloroquine, pyrimethamine, sulfadoxine, and quinine.

The results (Table I) indicate that the new derivatives, like the parent agents 1 and 2, are not cross-resistant to any of the antimalarial agents mentioned above. The derivatives are, in general, more effective against the W-2 than the D-6 strain. Esters (5a-d) possess activity comparable to that of the parent compounds, 1 and 2, although activity decreases as the aliphatic chain is elongated from one (5a) to three carbons (5c). Conversion of the esters (5a-d) to their corresponding carboxylates (7a-c) or acids (8b-d), with the exception of 7d and 8d (artelinic acid), drastically decreases the antimalarial activities in both cell lines.

Overall, the free acids exhibit better in vitro antimalarial activities than their salts; however, 7d, the salt form of 8d, is not only water soluble and stable in solution but also possesses comparable in vitro activity to artesinin (1). Because artelinic acid appears to be the most promising of the series, further studies on its in vivo antimalarial activity were assessed in a Plasmodium berghei mouse.

### Table I. In Vitro Antimalarial Activities against P. falciparum

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage</th>
<th>Vehicle</th>
<th>SIerra Leone (D-6)</th>
<th>Indochina (W-2)</th>
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<tr>
<td></td>
<td>µg/mL</td>
<td></td>
<td>IC₅₀ ng/ml</td>
<td>IC₅₀ ng/ml</td>
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<tr>
<td>artesinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>6.64</td>
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<td></td>
<td>0.41</td>
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<td>5a</td>
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<td></td>
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<tr>
<td>5b</td>
<td></td>
<td></td>
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<td>1.06</td>
</tr>
<tr>
<td>5c</td>
<td></td>
<td></td>
<td>3.00</td>
<td>0.95</td>
</tr>
<tr>
<td>5d</td>
<td></td>
<td></td>
<td>0.77</td>
<td>0.03</td>
</tr>
<tr>
<td>7a</td>
<td></td>
<td></td>
<td>53.79</td>
<td>26.23</td>
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</table>

### Table II. Antimalarial Activity of Artelnic Acid (8d) and Related Compounds against P. berghei in Mice

<table>
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<th>Compound</th>
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<th>No. of Cures</th>
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</thead>
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<td>artesinin (4)</td>
<td>640</td>
<td>NaHCO₃</td>
<td>5/5</td>
</tr>
<tr>
<td>artesinin (1)</td>
<td>160</td>
<td>NaHCO₃</td>
<td>5/5</td>
</tr>
<tr>
<td>artesinin (3)</td>
<td>160</td>
<td>NaHCO₃</td>
<td>3/5</td>
</tr>
<tr>
<td>artesinin (4)</td>
<td>40</td>
<td>NaHCO₃</td>
<td>2/5</td>
</tr>
</tbody>
</table>

Notes

* * A = active. The terms cure and active are defined in the Experimental Section.
screen side-by-side with the reference compounds, arteminisidin (1) and artemesic acid (3). The results are shown in Table II.

At a high dose level (640 mg/kg), all three compounds, 1, 3, and 8d, showed complete cures in the mice treated (5/5). At a lower dose level (160 mg/kg), artemesic acid was active, but there were no 60-day survivors among the treated mice. Artemesin and artemesic acid showed 5/5 cures at the same dose level. However, at the lowest dose level of 40 mg/kg, artemesin acid still showed a 100% cure, whereas artemesin showed 3/5 active and 2/5 cure. In contrast, none of the mice treated with artemesic acid showed 60-day survival. The results indicate that the new analogue 8d possesses superior in vivo activity against P. berghei than artemisinin or artemesic acid.

The stability studies performed in 2.5% K2CO3/D2O revealed that no detectable changes occur in 8d after 35 days and <3% hydrolyzed after 72 days at room temperature, whereas substantial hydrolysis (20%) took place in 3 under identical conditions within 1.5 h, with nearly complete hydrolysis taking place after 4 days (Table III). The rate of hydrolysis of 3 in 5% NaHCO3/D2O solution was found to be slower than in 2.5% K2CO3/D2O. The half-life was estimated to be about 4.5 days in 5% NaHCO3 (Table IV) compared to <1 day in 2.5% K2CO3 solution.

The superior in vivo antimalarial activity and stability in aqueous solution of 8d to artemesic acid renders the new compound a potential candidate drug for the treatment of cerebral malaria.

**Experimental Section**

**Chemistry.** All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr discs on a Perkin-Elmer Model 237 spectrophotometer. NMR spectra were run on a JOEL FX90Q spectrometer using MeSi as an internal standard. Analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI.

**Dihydroartemisinin (2).** Artemisinin (1; 0.5 g, 1.8 mmol) in 40 mL of MeOH was cooled in an ice bath to 0-5°C. To the solution was added in small portions 0.25 g (6.6 mmol) of NaBH4 over a period of 30 min. The solution was stirred at 0-5°C for 2 h after the addition of NaBH4 was complete, and the solution was neutralized with 30% AcOH/MeOH and evaporated to dryness under reduced pressure. The residue was extracted three times with 50 mL of Et2O. The Et2O extracts were combined, filtered, and evaporated to dryness to give 0.98 g (75%) of white needles. mp 152-154°C. Recrystallization from EtOAc/hexane raised the melting point to 153-155°C (lit.3 mp 153-154°C).

**Ethyl 2-(10-Dihydroartemisininoxynoyl)acetate (5a).** Dihydroartemisinin (2; 0.5 g, 1.75 mmol) was dissolved in 70 mL of anhydrous Et2O. To the solution were added successively 0.5 g (5 mmol) of ethyl glycolate and 0.25 mL of HF/ Et3O. The reaction mixture was stirred at room temperature for 24 h, washed successively with 5% aqueous NaHCO3 and H2O, dried over Na2SO4, and evaporated to dryness under reduced pressure. The resultant oil was purified by preparative TLC using EtOAc/hexane (1:3, v/v) to give 0.45 g (68%) of 5a; mp 50-52°C (IR neat) 1725 cm-1 (OC=O), 1587 cm-1 (OC=O) and 1040 cm-1 (C=O), 1H NMR (CDCl3) δ 6.75 (d, J = 3.6 Hz, 1 H), 7.03 (d, J = 7.2 Hz, 2 H), 7.28 (m, 1 H), 4.20 (t, at J = 7.2 Hz, 2 H), 3.85 (t, J = 7.2 Hz, 4 H), 3.78 (s, 3 H), 3.65 (s, 3 H), 2.97 (t, J = 4.8 Hz, 2 H), 1.77 (t, J = 4.8 Hz, 2 H), and 1.43 (s, 3 H), 0.95 (d, J = 6.3 Hz, 3 H), and 0.87 (d, J = 7.2 Hz, 3 H). Anal. Calcd for C20H20O9: C, 62.50; H, 4.93. Found: C, 62.99; H, 4.89. The minor product (22% yield) with a higher Rf value than 5a, was identified by NMR as the ethyl ether of 2 (6b, artether)16. 1H NMR (CDCl3) δ 6.39 (d, J = 7.2 Hz, 3 H), 0.76 (t, J = 3.6 Hz, 3 H), 1.08 (d, J = 7.2 Hz, 3 H), 1.52 (t, J = 4.8 Hz, 2 H), 4.07 (d, J = 1.2 Hz, 2 H), 3.85 (t, J = 4.8 Hz, 2 H), and 3.78 (s, 3 H). 13C NMR (CDCl3) ppm 128.5, 57.6, 56.4, 42.1, 41.3, 37.6, 36.5, 25.8, 24.1, 20.6, 18.6, 15.9, 2.9, and 0.95 ppm.

**Methyl 1-(10-Dihydroartemisininoxynoyl)butyrate (5c).** The same procedure for the preparation of 5a was used to prepare 5b by treating 0.5 g (1.75 mmol) of 2 with 1 g (9.6 mmol) of methyl 3-hydroxypropionate17 (1 g, 9.6 mmol). It yielded 46.5% (70%) of the desired product as an oil after purification by preparative TLC (silica gel, EtOAc/hexanes, 1:2, v/v). The compound solidified on standing: mp 76-78°C; IR (neat) 1743 cm-1 [OC=O]. 1H NMR (CDCl3) δ 5.43 (s, 1 H), 4.80 (d, J = 3.3 Hz, 1 H), 4.10 (m, 1 H), 3.68 (s, 3 H), 3.67 (m, 1 H), 2.58 (t, J = 4.4 Hz, 2 H), 1.44 (s, 3 H), 0.95 (d, J = 6.3 Hz, 3 H), and 0.87 (d, J = 7.2 Hz, 3 H). Anal. Calcd for C12H14O5: C, 62.51; H, 4.89. Found: C, 62.56; H, 4.83. The minor product, with a higher Rf value than 5c (22%), was identified by NMR as the methyl ester of 2 (6a, artemether). 1H NMR (CDCl3) δ 5.90 (d, J = 6.3 Hz, 3 H), 0.96 (d, J = 7.2 Hz, 3 H), 1.44 (s, 3 H), 2.6 (m, 1 H), 3.42 (s, 3 H), 4.68 (d, J = 3.6 Hz, 1 H), and 3.58 (s, 1 H). 13C NMR (CDCl3) ppm 124.5, 20.9, 24.99, 24.70, 26.22, 30.93, 34.67, 36.47, 37.51, 43.47, 52.60, 55.56, 81.10, 85.76, 106.33, 104.79.

**Methyl [1-(10-Dihydroartemisininoxynoyl)methyl]benzoate (5d).** Treatment of 1 g (6.0 mmol) of methyl p-hydroxybenzoate18 with 0.5 g (1.75 mmol) of 2 gave 70% of the desired product as an oil after purification: IR (neat) 1740 cm-1 [OC=O]; 1H NMR (CDCl3) δ 5.98 (s, 1 H), 1.77 (d, J = 3.6 Hz, 1 H), 3.85 (m, 1 H), 3.34 (m, 1 H), 1.43 (s, 3 H), 0.96 (d, J = 3.6 Hz, 3 H), and 0.90 (d, J = 7.2 Hz, 3 H). Anal. Calcd for C17H18O5: C, 62.50; H, 4.93. Found: C, 63.13; H, 4.86.

**Potassium 2-(10-Dihydroartemisininoxynoyl)acetate (7a).** Ester 5a (0.24 g, 0.65 mmol) was dissolved in 10 mL of 2.5% KOH/MEOH solution and allowed to stand at room temperature for 2 days. The solvent was reduced to half volume and diluted with an equal volume of H2O. The solution was passed through a reverse-phase column [EM Reagents, Lobar prepacked column size B (310-25). Lichroprep RP-8, 40-63 nm] and eluted with MeOH + H2O (1:1, v/v). The fractions were monitored by reverse-phase TLC (MeOH, ethyl acetate, and hexane). Evaporation of the desired compound were pooled, and the MeOH was evaporated under the reduced pressure. The aqueous solution was lyophilized to give 0.15 g (62%) of white crystals of 7a, mp 157-159°C. Anal. (C17H16O2·K·H2O) C, H, O.

Prepared by the same procedure were the following compounds: Potassium 2-(10-Dihydroartemisininoxynoyl)propionate (7b, 78%), mp 154°C dec. Anal. (C16H14O2·K· H2O) C, H, O.

Potassium 4-(10-Dihydroartemisininoxy)butyrate (7c; 40%; mp 142 °C dec). Anal. (C₉H₁₇O₃K·H₂O) C, H.

Potassium p-[4(10-Dihydroartemisininoxy)methyl]benzoate (7d; 60%; mp 158°C dec). Anal. (C₁₉H₂₁O₅K·H₂O) C, H.

3-(10-Dihydroartemisininoxy)propionic Acid (8b). Ester 5b (0.4 g, 1 mmol) in 10 mL of 2.5% KOH/MeOH solution was allowed to stand at room temperature for 2 days. The solvent was then evaporated to dryness under the reduced pressure. The residue was dissolved in 10 mL of H₂O and the solution was washed twice with an equal volume of Et₂O. The aqueous layer was acidified with AcOH and the mixture was extracted two times with Et₂O. The Et₂O extracts were combined, dried over Na₂SO₄, and evaporated to dryness. The oily product crystallized from hexane/Et₂O to give white crystals (0.25 g, 70%): mp 160-162 °C; IR (KBr) 1740 cm⁻¹ (COOH);¹ H NMR (CDCl₃) δ 0.88 (d, J = 7.2 Hz, 3 H), 0.94 (d, J = 3.6 Hz, 3 H), 1.44 (s, 3 H), 2.62 (t, J = 6.3 Hz, 2 H), 3.63 (m, 1 H), 4.14 (m, 1 H), 4.82 (d, J = 3.6 Hz, 1 H), 5.45 (s, 1 H), and 7.99 (d, J = 3 Hz, 1 H). Anal. (C₁₉H₂₁O₄) C, H.

4-(10-Dihydroartemisininoxy)butyric Acid (8c). The same procedure for the preparation of 8b was used to prepare 8c from 5c in 60% yield. The product is an oil: 1H NMR (CDCl₃) δ 0.88 (d, J = 7.2 Hz, 3 H), 0.96 (d, J = 3.6 Hz, 3 H), 1.43 (s, 3 H), 3.39 (m, 1 H), 3.90 (m, 1 H), 4.79 (d, J = 3.6 Hz, 1 H), and 5.39 (s, 1 H). Anal. (C₁₉H₂₁O₄) C, H.

p-[4(10-Dihydroartemisininoxy)methyl]benzoic Acid (8d, Arterlineic Acid). The same procedure for the preparation of 8b was adapted to the preparation of compound 8d from 5d in 55% yield. Purification was achieved by recrystallization from MeOH/H₂O. mp 142-145 °C; IR (KBr) 1700 cm⁻¹ (COOH);¹ H NMR (CDCl₃) δ 0.96 (d, J = 7.2 Hz, 3 H), 0.98 (d, J = 7.2 Hz, 3 H), 1.46 (s, 3 H), 2.71 (m, 1 H), 4.06 (d, J = 13.5 Hz, 1 H), 4.94 (d, J = 2.7 Hz, 1 H), 5.00 (d, J = 13.5 Hz, 1 H), 5.46 (s, 1 H), 7.42 (d, J = 8.1 Hz, 2 H), and 8.10 (d, J = 8.1 Hz, 2 H). Anal. (C₁₉H₂₁O₄) C, H.

Stability Studies of Arterlineic (8d) and Artesunic Acid (3). Acids. To a 5-mm NMR tube containing 10 mg of sample was added 0.5 mL of 2.5% K₂CO₃/D₂O solution. The initial spectrum of the solution was taken within 5 min and again at intervals thereafter (cf. Table III). The extent of hydrolysis was estimated by the following equation:

\[
\text{% decomposition} = \left(\frac{B - 2A + B}{X \times 100}\right)
\]

where \(A = \text{integration of H-10 (5.80 ppm, d, J = 10.8 Hz, 1 H) and H-12 (5.71 ppm, s, 1 H) of artemusate and B = integration of the singlet at 2.42 ppm for the potassium succinate formed. With time, the intensity of potassium succinate (2.42 ppm) signal increases, while that of H-10 and H-12 of artemusate decreases. The stability study of 3 in 5% NaHCO₃/D₂O was carried out by the same procedure.

Biology. (a) In Vitro Antimalarial Studies. The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al. and Milhous et al. Two P. falciparum malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (1-6), were utilized in susceptibility testing. They were derived by direct visualization and microinoculation from patient isolates obtained by the Centers for Disease Control, Atlanta, GA, in 1980 and 1982, respectively. The patients had acquired infections either in Vietnam or in Sierra Leone. The Indochina clone is resistant to the antimalarial chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone clone is resistant to mefloquine but susceptible to chloroquine, quinine, sulfadoxine, and pyrimethamine. Test compounds were initially dissolved in DMSO and 70% ethanol and diluted in RPMI 1640 culture medium with 10% human plasma to 400-fold. Drugs were subsequently further diluted by using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of (1.56-100) X 10⁻⁶ M. Parasite inocula (at 0.5% parasitemia and a 1% hemocrit) were incubated for 24 h and added to equimolar concentrations of each test compound prior to the addition of [³H]hypoxanthine. After a further incubation of 18 h, parasite matter was harvested from each microtiter well by using an automated cell harvester (Skatron, Inc., Sterling, VA). Uptake of [³H]hypoxanthine was measured by using a scintillation spectrophotometer (Model LS9801, Beckman Instruments, Irvine, CA). Concentration–response data were analyzed by nonlinear regression and the IC₅₀ values (50% inhibitory concentrations) for each compound were calculated.

(b) In Vivo Antimalarial Studies. The suppressive blood schizonticidal and curative activities of artesiminin, artesunic acid, and artesunic acid were measured in a test where mice were infected with 5.89 x 10⁶ P. berghei parasitized cells intraperitoneally on day 0. Test compounds were dissolved in either peanut oil or 5% NaHCO₃ aqueous solution and were administered subcutaneously once a day for 3 consecutive days commencing on day 3. The dose levels of compounds given were 640, 160, and 40 mg/kg per day. Blood films were taken on days 6, 13, and 20. Blood schizonticidal activity was determined by monitoring blood films for the appearance of parasites and for extended survival times compared to infected untreated controls. Mice surviving 60 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds was considered active when the survival time of the treated mice was greater than twice the control mice, i.e., 12–14 days.

Acknowledgment. We thank Dr. A. M. J. Oduola of the Department of Parasitology for preparing the clones for in vitro testing and Dr. Arba L. Ager, Jr., University of Miami, for performing the in vivo antimalarial studies.


(16) Oduola, A. M. J.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. Presented at the 32nd Annual Meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, TX, Dec 4–8, 1983; Abstract 58.
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