The chemotherapy of rodent malaria. XLVIII. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 1: Studies leading to the development of novel cis-fused cyclopenteno derivatives

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Abstract

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Reference


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The chemotherapy of rodent malaria. 
XLVIII. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 1: Studies leading to the development of novel cis-fused cyclopenteno derivatives*

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The new Chinese antimalarial blood schizontocide, artemisinin, derived from the plant Artemisia annua, displays a high level of activity against polyresistant Plasmodium falciparum. Several synthetic 1,2,4-trioxanes were examined in a search for compounds that exhibit a similar type of action against drug-resistant parasites. This paper, the first of a series, describes the examination of these trioxanes against drug-sensitive and drug-resistant malaria parasites in a rodent model, using artemisinin and arteether as comparison standards. Cis-fused cyclohexeno-1,2,4-trioxanes (10-17) substituted with various side-chains revealed for the most part variable but weak antimalarial activity. On the other hand, cis-fused cyclopenteno-1,2,4-trioxanes (18-19) showed greater activity, 19 showing about 1/30th of the activity of arteether against drug-sensitive Plasmodium berghei in vivo, thereby providing a clue to the structure-activity relationship.

The malignant tertian malaria parasite of man, Plasmodium falciparum, possesses a number of efficient genetic mechanisms that have permitted it to evolve resistance to almost all antimalarial drugs that are presently available for its prevention or treatment (Wellens, 1991). These drugs include the 4-aminoquinoline schizontocide chloroquine, the aminoalcohols quinine, mefloquine and halofantrine, and the so-called antifols sulfadoxine, proguanil and pyrimethamine that block folate metabolism. Moreover, many parasites now display polyresistance, i.e. they are resistant to more than one of these groups of compounds (Peters, 1990). In the past
two years chloroquine resistance has made its appearance also in *P. vivax* (although so far only in West Irian and Papua New Guinea). The diminishing effectiveness of the aforementioned drugs makes the search for compounds in new chemical classes, and possibly ones possessing novel modes of action against the parasites, an urgent matter.

Only two such classes of antimalarials have reached the stage of clinical trials in recent years. The first class is based on the sesquiterpene lactone, artemisinin (1, Scheme 1), the active principle of *Artemisia annua* Linn. (Compositae), a herb that has long been renowned in traditional Chinese medicine for its potent antipyretic properties (Peters, 1987). During the past decade artemisinin has become well established as a specific antimalarial blood schizontocide, particularly in China. Artemisinin and its semi-synthetic derivatives, artether (2), artemether (3), sodium artemisunate (4) and sodium artelinate (5, Scheme 1) show considerable promise for the treatment of infection with polyresistant *P. falciparum*, as well as infection with other malaria parasites of man. The second class is represented by the naphthoquinone derivative BWS66C (6, Scheme 1) which is currently in the early stages of clinical trial. Both these classes of compound appear, so far, to be active against the major target organisms, polyresistant strains of *P. falciparum*.

Artemisinin and its derivatives are relatively non-toxic, rapidly-acting blood schizontocides, the mode of action of which is presently unknown. These first-generation derivatives possess inherent disadvantages such as poor solubility in some cases and rapid metabolism of the peroxide function *in vivo* to yield inactive metabolites. Furthermore, since their total synthesis is impractical, they have to be obtained by derivitization of artemisinin, itself extracted from the plant with all the practical drawbacks that this procedure entails. The artemisinin molecule contains the 1,2,4-trioxane entity which appears to be necessary for its antimalarial activity.

Instead of devising new ways to synthesize artemisinin, our approach has been to identify the pharmacophore and to embody it in simpler molecules which are amenable to chemical modification. Accordingly, new methods have been developed for preparing simple trioxanes that retain the rapid blood schizontocidal activity of artemisinin. The result has been the discovery of cis-fused bicyclic 1,2,4-trioxanes that are the subject of the present study. Details of the early chemical approach were presented by Jefford (1991) and a preliminary note on the present work has been published by Jefford *et al.* (1988). This paper amplifies early studies that have led to a promising new class of 1,2,4-trioxane antimalarials.

**MATERIALS AND METHODS**

The procedures employed, which have been published in detail elsewhere (Peters, 1987), are briefly summarized here.

**Parasite Species and Lines**

The Keyberg 173 N strain of *P. berghei*, which is sensitive to all currently used antimalarial
TABLE 1
Drug-sensitive and drug-resistant lines used for drug evaluation

<table>
<thead>
<tr>
<th>Primary resistance</th>
<th>Plasmodium berghei-derived Line</th>
<th>P. yoelii NS-derived Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>N 1.0</td>
<td>NS 18.0</td>
</tr>
<tr>
<td>Quinine</td>
<td>Q 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>N/1100 120</td>
<td>—</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>—</td>
<td>ART 16.5</td>
</tr>
<tr>
<td>Primaquine</td>
<td>P 15</td>
<td>—</td>
</tr>
<tr>
<td>Sulphaphenazole</td>
<td>ORA 6.6</td>
<td>—</td>
</tr>
</tbody>
</table>

*Approximate levels of resistance to the compound against which resistance has developed, compared with the response of the P. berghei parent line (I₅₀ = ED₅₀ resistant line/ED₅₀ parent line (3.1 mg/kg sc daily × 4)).
†These I₅₀ values are calculated by comparing the ED₅₀ to chloroquine with that of P. yoelii nigeriensis (6.7 mg/kg sc daily × 4).
‡I₅₀ to sulfadoxine.

drugs, is used as an indicator of baseline responses to new test compounds. Plasmodium yoelii ssp. NS (the 'P. berghei NS' line of Peters et al., 1978) is an inherently chloroquine-resistant parasite that we consider to be a valuable model for naturally occurring chloroquine-resistant P. falciparum (Peters et al., 1975). Further data on the various drug-resistant lines shown in Table 1 derived from these parasites are given by Peters and Robinson (1992).

Test Procedures
MOUSE STRAIN AND HANDLING
Random bred, female Swiss mice free of contaminating organisms (such as Eperythrozoon coccoides) weighing 20 ± 2 g are fed on a standard diet with water ad libitum in cages holding five animals each. The environment is maintained at a temperature of 22°C and 80% relative humidity.

PREPARATION OF COMPOUNDS
Many of the synthetic trioxanes examined proved to be poorly soluble or insoluble in water. They were therefore suspended with the aid of ultrasonication either in water or saline containing Tween 80 or in a 10% solution of dimethylsulphoxide (DMSO) in water.

‘FOUR-DAY TEST’ OF BLOOD SCHIZONTOCIDAL ACTIVITY
The mice are infected intravenously (iv) via the dorsal tail vein on the day of infection DO with approximately 10⁸ infected donor erythrocytes. Drugs are administered subcutaneously (sc) or orally (po) later on DO, then daily for three more days (D + 1 through D + 3). Parasitaemia levels are assessed on Giemsa-stained thin blood films made from the tail vein on D + 4.

RESULTS
Blood Schizontocidal Action of Ascaridole (7), Trioxadecalin (9) and Dihydropyridophosphorane (1,2,4-trioxines (10–17)
In the preliminary study only the drug-sensitive P. berghei N strain was used. One of the first compounds selected was the anthelminthic drug ascaridole (7, Scheme 2) which contains a simple peroxide function that we thought might endow it with an artemisinin-like activity. Next we examined a monocyclic 1,2,4-trioxane bearing a 2-methylphenylamino substituent attached at the C5 atom (8). Ascaridole proved to be toxic to the hosts at 10 mg/kg at which dose it was entirely inactive (Table 2). Trioxane (8) showed a low level of activity with an ED₅₀ of...
Scheme 2. Compounds 7 to 9.

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound nos</th>
<th>Name</th>
<th>$ED_{50}$ (mg/kg/day sc × 4)</th>
<th>$ED_{90}$</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Artemisinin</td>
<td>2.5</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Arteether</td>
<td>0.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ascaridole</td>
<td>&gt;MTD</td>
<td>&gt;MTD</td>
<td>MTD 10 mg/kg * Maximum dose tested</td>
</tr>
<tr>
<td>8</td>
<td>Trioxadecalin</td>
<td>60</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>30</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>45</td>
<td>330**</td>
<td>** Interpolated graphically</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>120</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>42</td>
<td>540**</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>80</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>42</td>
<td>770**</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>&gt;100</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>&gt;100</td>
<td>&gt;100*</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>8</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>12.8</td>
<td>24.5</td>
<td>$ED_{50}$ and $ED_{90}$ po 10.5 and 22.0</td>
</tr>
<tr>
<td>Chloroquine</td>
<td></td>
<td>1.8</td>
<td>3.2</td>
<td>As diphosphate</td>
</tr>
</tbody>
</table>

N.A., Not active.

Scheme 3. Compounds 10 to 12.
about 60 mg/kg, whereas the trans-fused bicyclic trioxane 9 was about twice as active at the ED₅₀ level.

From this promising beginning, two further series of compounds based on the 4a,10b-dihydro-6,10b-dimethylnaphtho[2,1-e][1,2,4-trioxane] entity were examined. The first series are substituted at the C₃ position with a methyl, hydroxymethyl or hydroxyethyl group (compounds 10, 11 and 12). They were all mixtures of the C₃ epimers. The second series, derived from 11 and 12, are carboxamides (13, 14), benzoates (15, 16) and a phenylacetate (17). They too are epimeric mixtures. Of the first series, the highest level of activity was observed in 10 and 12 with poor activity in 11. Two of the second series showed low level activity (13, 14) whereas 15 was totally inactive. The long chain benzoate 16 and the acetate 17 showed only marginal activity at the maximum doses tested.

Blood Schizontocidal Action of Cis-fused Cyclopenteno-1,2,4-trioxanes
These findings revealed that no extra antimalarial activity arises on attaching side-chains to the cis-fused dihyronaphththeno-1,2,4-

DISCUSSION
None of the compounds described here displayed the high levels of activity that are exhibited by artemisinin or arteether. The
results demonstrate, nonetheless, that the 1,2,4-trioxane ring is part of the pharmacophore, but that it is not sufficient in itself to ensure artemisinin-like activity. However, the net improvement in activity arising from the cis-fused cyclopentene over the six-membered naphthalene ring (e.g. 19 vs. 10) is noteworthy.

Having in hand the technology for preparing a wide range of trioxanes, further alterations in structure which build on these early findings are feasible. Such studies are under way. The preparation and testing of the compounds so obtained will be described in subsequent papers in this series.

REFERENCES


