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Dispiro-1,2,4,5-tetraoxanes: A New Class of Antimalarial Peroxides

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Dispiro-1,2,4,5-tetraoxanes 2-4 were synthesized as potential peroxide antimalarial drugs. They had curative activity against Plasmodium berghei in vivo at single doses of 320 and 640 mg/kg which confirms earlier unpublished data. Moreover, artemisinin (1) and 4 had equivalent ED₅₀'s against P. berghei in vivo in the multiple-dose Thompson test; neither showed any evidence of acute toxicity at total doses of more than 12 g/kg. Dispiro-1,2,4,5-tetraoxane 4 had IC₅₀'s comparable to those of 1 against Plasmodium falciparum clones in vitro. These results confirm the potential of dispiro-1,2,4,5-tetraoxanes as a new class of inexpensive peroxide antimalarial drugs.

As a result of an apparent association between the peroxide functional group and antimalarial activity, a substantial effort has been devoted to developing new peroxide antimalarials. Our early attempts in this regard led us to conclude that an endoperoxide ketal is a minimum but insufficient structural requirement for an effective peroxide-containing antimalarial. Most work, however, has centered around artemisinin (1), the proto-


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type peroxy antimalarial, in an effort to discern its structure-activity relationship (SAR). These investigations identify the 1,2,4-trioxane heterocycle as the critical

(4) We found that two tricyclic peroxy ketals (7-acetyl-1,4,6-trimethyl-2,3,5,10-tetraoxatricyclo[4.3.1.0'2.6]decane and 1,4,6-trimethyl-2,3,5,7,8-pentaoxatricyclo[4.2.2.0'6.10]decane) described by Bischoff, C.; Riecle, A. Uber die Bildung cyclischer Peroxide aus Mehrfachketten. Liebig Ann. Chem. 1969, 725, 87-92, have IC₅₀ values >300 nM against \( P. falciparum \) in vitro and are inactive against \( P. berghei \) with toxicity observed at a 640 mg/kg dose. We thank Prof. Christophe Morin, of the Universite de Grenoble, St.-Martin D’Heres, France for bringing these peroxides to our attention as potential antimalarials.

(a) Acton, N.; Klayman, D. L. Conversion of Artemisin C values for 1 represent averages of eight data sets. 


![Figure 1](https://example.com/figure1.png)

**Figure 1.** Dose-response curves for 1 (hatched line) and 4 (solid line) in the Thompson test. Doses of 1 and 4 were administered sc twice daily for 3 days, starting at day 3 postinfection.

Others have synthesized structurally diverse 1,2,4-trioxanes which show mixed results when screened for pharmacopephty, and suggest that an intact ABC ring system is required for maximum potency.

Table I. Antimalarial Activity of Dihydropyr-1,2,4,5-tetroxanes 2-4 Compared to Artemisinin (1)

<table>
<thead>
<tr>
<th>compd</th>
<th>D-6</th>
<th>W-2</th>
<th>resistance indexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7</td>
<td>2.2</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>13</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>8.2</td>
<td>58</td>
<td>0.71</td>
</tr>
<tr>
<td>4</td>
<td>6.9</td>
<td>3.4</td>
<td>0.49</td>
</tr>
</tbody>
</table>

\( T - C \) is the mean survival time of the treated mice beyond that of the control animals. This value must be \( \geq \) twice the mean survival time (6.2 days) of the control animals to be considered active (A). Survival beyond 60 days is considered curative (C), and deaths from 0-2 days postinfection are attributed to toxicity (T). \( IC₅₀ \) values. 

- **Table 1**

<table>
<thead>
<tr>
<th>( P. falciparum ) IC₅₀ (mM)</th>
<th>( P. berghei ) T - C* (days) at the following doses (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>compd</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* IC₅₀ (W-2)/IC₅₀(D-6) ratio. Single dose administered sc 3 days postinfection, \( n = 5. \) 

**IC₅₀** values for 1 represent averages of eight data sets from WRAIR. * Unpublished data. 


antimalarial activity. For example, Kepler et al. synthesized 10 1,2,4-trioxanes that were inactive against P. berghei in vivo; the most active compounds in this series had IC50s between 24 and 100 ng/mL against P. falciparum in vitro. Singh et al. synthesized several 1,2,4-trioxanes with IC50s ranging from 3 to 222 ng/mL against P. falciparum in vitro. Jefferd et al. reported that 10 1,2,4-trioxanes were either inactive or much less active than was 1 against P. berghei in mice. More recently, however, Jefferd et al. described excellent in vitro and in vivo antimalarial activity for numerous 1,2,4-trioxanes, many of which were more potent than 1.

It must be noted that 1 is curative against P. berghei only at 640 mg/kg in the single-dose Rane screen (Table I); its curative activity improves dramatically, however, when a multiple-dose protocol is followed. In this light, unpublished data indicates several dispiro-1,2,4,5-tetraoxanes are curative at single doses of 320 and 640 mg/kg is remarkable. We now confirm this curative in vivo activity and show that dispiro-1,2,4,5-tetraoxanes are also active against P. falciparum in vitro.

Chemistry

From this unpublished data, we chose three of the more active dispiro-1,2,4,5-tetraoxanes (2-4) which were obtained via acid-catalyzed peroxyketalization between a 1:1 mole ratio of the substituted cyclohexanone and hydrogen peroxide. A modified procedure of Braunworth and Crosby using 30% H2O2 and H8O4 in aqueous EtOH at 0 °C afforded 2 and 3,16,18 We found the procedure of Sanderson et al. using 30% H2O2, HCl, and HClO4 in acetic acid at room temperature to be the best method for the synthesis of 4.1,18 1H and 13C NMR spectroscopy indicate that 2 and 3 are each mixtures of meso and d,l stereoisomers, whereas 4 is a single stereoisomer. For example, 4 has a seven-line 13C NMR spectrum consistent with the presence of a single stereoisomer, whereas 2 and 3 each have 2-3-fold the number of lines expected for that of a single diastereomer. Furthermore, 1H NMR spectra show a single doublet for the methyl groups in 4, and closely overlapping doublets for the methyl groups in 2 and 3. Previous X-ray crystallographic structural analysis of 418 has shown that it exists as a single meso stereoisomer.

Antimalarial Activity

In vivo antimalarial activity for 2-4 against a drug-sensitive strain (KBBG 173) of P. berghei in the single-dose Rane screen was comparable (Table I) to unpublished data with cures seen at doses of 320 and 640 mg/kg. A multiple-dose Thompson test against P. berghei (Figure 1) revealed no significant difference between ED50's of 1 (607 mg/kg) and 4 (382 mg/kg) at the 95% confidence intervals using nonlinear regression. Both drugs demonstrated a remarkable lack of acute toxicity; no deaths were observed at total doses of more than 12 g/kg.

In vivo antimalarial activity against the D-6 and W-2 clones of P. falciparum (Table I) using a semiautomated microdilution technique indicates that 2 and 3 are 2- to 26-fold less potent than is 1; curiously, 5, in contrast to 1, 2, and 4, has a high resistance index. Dispiro-1,2,4,5-tetraoxane 4 is only 1.5-fold less potent than is 1 against both P. falciparum clones. The excellent potency of 4 against P. falciparum in vitro is consistent with its superior single-dose in vivo activity in comparison to 2 and 3. The range of in vitro potencies for 2-4 is also consistent with unpublished data of Doorenbos and Decker who noted considerable changes in in vivo antimalarial activity with relatively small structural modifications.

Discussion

Even though an overlay of energy-minimized structures of 1 and 42 using PCMODEL reveals substantial structural differences between 1 and 4, the relative antimalarial activity of 1 and 4 is consistent with the data that 1 is a single meso stereoisomer.


(21) Data from Walter Reed Army Institute of Research (WRAIR).

(22) Antimalarial data (P. berghei) from WRAIR indicates that several 1,2,4,5-tetroxanes derived from cyclic ketones are curative, whereas those derived from acyclic ketones and aldehydes are not. Cyclic triperoxides (1,2,4,5,7,8-hexaoxanes) which are kinetic products (Story, P. R.; Lee, B.; Bishop, C. E.; Denson, D. B.; Busch, P.). Macro cyclic Synthesis. II. Cyclohexanone Peroxides. J. Org. Chem. 1970, 35, 3059-3062) of acid-catalyzed peroxyketalization between hydrogen peroxide and ketones are without activity.


dicate that 4, like 1, exists as a relatively rigid structure. If one speculates that the 1,2,4,5-tetroxane in 1 corresponds with the 1,2,4,5-tetraoxan in 4, several correlations are evident: (1) the oxygen-substituted carbon atom (C-12) in 1 is replaced with an oxygen atom in 4; (2) the C-3 methyl and 4,6-ethano bridge (which forms the A-ring in 1) is replaced with a methyl-substituted spirocyclohexane in 4; and (3) the C-ring, a substituted spirocyclohexane with respect to the 1,2,4-tetroxane (B-ring) in 1, may correspond to the other spirocyclohexane ring in 4. These correlations are consistent with the observation that full potency is seen with certain ABC ring analogs of 1,2,4,5-tetraoxan.

As the peroxide bond is absolutely essential for antimalarial activity in 1, it is likely that at least one of the peroxide bonds in 4 is required. To partially address this question, work is in progress to screen the analogous dispiro-1,2,4,5-tetraoxanenes and dispiro-1,2,4,5-tetraoxananes which differ from dispiro-1,2,4,5-tetraoxanenes by removal of one oxygen atom by replacement of an oxygen atom by a methylene carbon, respectively.

In summary, we suggest that the potency observed with 4 against P. falciparum in vitro confirms the potential of dispiro-1,2,4,5-tetraoxanenes as a new class of peroxide antimalarial drugs. An expected advantage of these compounds with respect to 1 and its semisynthetic derivatives is a one-step synthesis using inexpensive starting materials vs a multistep synthesis or isolation from Artemesia annua. Work is in progress to synthesize additional dispiro-1,2,4,5-tetraoxanenes to define SAR and optimize antimalarial activity.

Experimental Section

Molecular modeling experiments were performed with PCMODEL 4.0 (Serena Software). Melting points were taken with a Melt-Temp capillary apparatus. IR spectra were run as KB or discs on a Perkin Elmer 1420 spectrophotometer. NMR spectra were obtained with Varian XL-300 or Bruker AC-200 spectrometers using deuterated chloroform and TMS as an internal standard. Microanalyses were performed by M-H-W Laboratories, Phoenix, AZ. Analytical HPLC of 2-4 was performed on a 5-µm silica column (Alltech Spherisorb 320 x 4.6 mm) using a 97:3 heptane-tetra-butyl methyl ether mobile phase with detection at 220 nm. All reagents are commercially available from Aldrich Chemical Co. with the exception of 3,5-dimethylcyclohexanone. The latter was prepared by Jones oxidation of 3,5-dimethylcyclohexanol.

Synthesis

2,4,11,13-Tetramethyl-7,8,15,16-tetraoxodispiro[5.2.5.2]hexadecanone (2). Thirty percent H2O2 (23.3 mmol, 0.810 g) and 3,5-dimethylcyclohexanone (23.3 mmol, 3.00 g) were added by consecutive dropwise addition to a stirred solution of water (25 mL), EtOH (27 mL), and H2SO4 (50 mL) at 0°C. Stirring was continued for 14 h at 0°C. The resulting white precipitate was filtered, washed with water, and air-dried to afford 2 (2.64 g, 78%). mp 172-173°C (CH3CN) (lit.22 mp 172-173°C; IR 2940, 2920, 2840, 1445 cm⁻¹; 'H NMR δ 0.45-0.75 (m, 2 H), 0.77-1.22 (m, 16 H), 1.49-1.53 (m, 8 H), 2.05-2.34 (m, 2 H); 13C NMR δ 21.87, 21.94, 22.02, 28.29, 28.63, 28.93, 36.40, 36.50, 40.12, 43.23, 43.38, 58.97, 105.06. Anal. (C19H24O4) C, H.

2,4,11,13-Tetramethyl-7,8,15,16-tetraoxodispiro[5.2.5.2]hexadecanone (3): 0.78 g, 70%; mp 71-72°C (84:MeOH/H2O) (lit.25,26 mp 71-72°C; IR 2950, 2930, 2860, 1445 cm⁻¹; 'H NMR δ 0.75-1.02 (m, 6 H), 1.03-1.20 (m, 14 H), 2.01-2.41 (m, 4 H); 13C NMR δ 21.51, 21.60, 21.83, 28.50, 28.57, 30.78, 30.93, 30.96, 31.06, 31.11, 31.16, 31.65, 31.73, 31.76, 31.83, 107.51, 107.58, 107.71. Anal. (C19H24O4) C, H.

2,4,11,13-Tetramethyl-7,8,15,16-tetraoxodispiro[5.2.5.2]hexadecanone (4). Thirty percent H2O2 (0.1 mol, 1.340 g) was added by slow dropwise addition to a stirred mixture of 2-methylcyclohexanone (0.1 mol, 11.22 g) and 2 N HCl (1 mL) at room temperature. Stirring was continued for 12 h at room temperature at which time glacial AcOEt (75 mL) and 10% HClO4 (4 mL) were added. Stirring was continued for an additional 12 h at room temperature. Water (150 mL) was added, and 4 (7.70 g, 60%) was collected as a white solid after filtration and washing with water: mp 105-106°C (MeOH) (lit.27 mp 105-106°C); IR 2940, 2860, 1460, 1440 cm⁻¹; 'H NMR δ 1.01 (d, J = 6.9 Hz, 6 H), 1.19-1.98 (m, 16 H), 2.73-3.05 (m, 2 H); 13C NMR δ 13.65, 22.46, 24.54, 29.41, 30.71, 38.21, 109.17. Anal. (C19H24O4) C, H.

Screening Methods. In vitro activity against P. falciparum was determined using a modification of the semiautomated microdilution technique of Desjardins et al.25 and Milhoue et al.21 Two P. falciparum malaria parasite clones, designated as Sierra Leone (D-6) and Indochine (W-2), were used in susceptibility testing. The former was resistant to mefloquine, and the latter to chloroquine (CQ), pyrimethamine, sulfadoxine, and quinine. Test compounds were dissolved in dimethyl sulfoxide and solutions serially diluted with culture media. Erythrocytes with a 30-40% parasitemia were added to each well of a 96-well plate. Stirring was continued for 14 h at 37°C in a 5% CO2 atmosphere. Results were reported as IC50 (ng/mL) values.

In vivo activity against P. berghei was obtained against a drug-sensitive strain of P. berghei (strain KBG 173).28 Each test compound was administered to five male mice per dilution in a single subcutaneous (sc) dose 5 days after infection. Results were expressed in T - C values which indicate the mean survival time.
of the treated mice beyond that of the control animals; untreated mice survive on average 6.2 days. Compounds were classified as active (A) when the mean survival time of the treated mice is twice that of the controls (>12.4 days), and curative (C) when one or more test animals live 60 days postinfection. Deaths from 0–2 days post-treatment were attributed to toxicity (T).

A slight modification of the Thompson test was used to further quantify antimalarial activity and toxicity. Five week old CD-1 mice were inoculated on day 0 with $5 \times 10^6$ trophozoites of P. berghei (strain KBG 173) obtained from an infected mouse at 60% parasitemia, diluted with uninfected mouse blood, and injected intraperitoneally. On days 3–5, each group of seven mice were treated sc with the compound to be tested in eight total doses, twice a day for 3 days. A range of doses sufficient to generate a dose-response curve was used. Blood films were taken 1 day after completion of drug treatment (day 6) and weekly thereafter until day 60. Parasitemia values were determined from Giemsa-stained blood films. Drug activity was evaluated by suppression of parasitemia, extension of survival time, and curative activity. Mice living 60 days postinfection and blood film negative were considered cured. A drug was considered to be toxic if the mice died before the untreated control mice. An advantage of this method of evaluation is the ability to assess efficacy and acute toxicity at the same time in the same model.

Acknowledgment. This work was funded in part by DHHS/NIH/NIAID Grant No. 1 R15 AI28012-01. Mass spectral determinations were performed by the Midwest Center for Mass Spectrometry, a NSF Regional Instrument Facility (CHE 8921777). We thank Professor Thomas R. Hoye of the University of Minnesota for helpful discussions on the stereochemistry of dispiro-1,2,4,5-tetraoxanes, and Dr. Peter R. Gwilt of the University of Nebraska Medical Center for nonlinear regression analysis of the Thompson test data.

Thieno[2,3-b]furan-2-sulfonamides as Topical Carbonic Anhydrase Inhibitors


Merck Research Laboratories, Departments of Medicinal Chemistry and Ocular Pharmacology, West Point, Pennsylvania 19486. Received March 9, 1992

Novel 5-[(alkylamino)methyl]thieno[2,3-b]furan-2-sulfonamides were prepared and evaluated in vitro for inhibition of human carbonic anhydrase II (CA II) and ex vivo for their ability to inhibit CA II in the albino rabbit eye after topical administration. Compound 11a was found to lower intraocular pressure (IOP) in both the α-CT ocular hypertensive albino rabbit and the normal albino rabbit, but was ineffective at lowering IOP in a hypertensive, pigmented monkey model. Since 11a was highly bound to ocular pigment, a series of less basic analogs was prepared. Examples in this series were both less extensively bound to ocular pigment and more active at reducing IOP in pigmented rabbits after topical dosing. Key examples displayed moderate reactivity toward glutathione.

Introduction

Topically effective carbonic anhydrase inhibitors that minimize systemic side effects are a potentially important therapeutic advance in the treatment of open-angle glaucoma. Recently, several novel classes of inhibitors, including sulfonamides of benzo[8]thiophene, benzo[b]-furan, 4-aminothienothiopyran, thieno[2,3-b]thiophene, and 5-[(hydroxalkyl)sulfonyl]thiophene, have been reported. Leading representatives in these classes demonstrate nanomolar level potency for inhibition of human carbonic anhydrase II (CA II) in vitro, ocular hypotensive efficacy in animals after topical dosing, minimal sensitization potential, and appropriate solubility at or near physiological pH to allow for dosing as a suspension or a solution. As part of our continuing work in this area we wish to report on the biological evaluation of a series of...