Spiroadamantyl 1,2,4-Trioxolane, 1,2,4-Trioxane, and 1,2,4-Trioxepane Pairs: Relationship Between Peroxide Bond Iron(II) Reactivity, Heme Alkylation Efficiency, and Antimalarial Activity

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Spiro adamantyl 1,2,4-trioxolane, 1,2,4-trioxane, and 1,2,4-trioxepane pairs: Relationship between peroxide bond iron(II) reactivity, heme alkylation efficiency, and antimalarial activity

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Abstract
These data suggest that iron(II) reactivity for a set of homologous spiro adamantyl 1,2,4-trioxolane, 1,2,4-trioxane, and 1,2,4-trioxepane peroxide heterocycles is a necessary, but insufficient, property of antimalarial peroxides. Heme alkylation efficiency appears to give a more accurate prediction of antimalarial activity than FeSO4-mediated reaction rates, suggesting that antimalarial activity is not merely dependent on peroxide bond cleavage, but also on the ability of reactive intermediates to alkylate heme or other proximal targets.

Keywords: 1,2,4-trioxolane, 1,2,4-trioxane, 1,2,4-trioxepane, peroxide, antimalarial, artemisinin

The semisynthetic artemisinins rapidly reduce parasite burden and are particularly effective when used in a 3-day artemisinin combination treatment (ACT) regimen. One hypothesis2–4 that accounts for the antimalarial specificity of artemisinin5 is that the peroxide bond undergoes reductive activation by heme released by parasite hemoglobin digestion to produce carbon-centered free radicals or carbocations that alkylate heme6 or parasite proteins. Although the 1,2,4-trioxane heterocycle in artemisinin (Figure 1) is the critical pharmacophore, its presence alone7 is insufficient for high antimalarial activity as most synthetic 1,2,4-trioxanes are less active than artemisinin.8 The discovery9 of ozonide (1,2,4-trioxolane) drug development candidate OZ277 (arterolane) (Figure 1), now entering Phase III clinical trials in the form of an arterolane malate + piperaquine phosphate combination, prompted us to investigate a set of structurally related 1,2,4-trioxolanes 1, 1,2,4-trioxanes 2, and 1,2,4-trioxepanes 3 (Figure 2) to better understand the relationship between peroxide bond iron(II) reactivity and antimalarial activity for these homologous peroxide heterocycles.

Trioxolanes 1b and 1c were obtained by Griesbaum coozonolysis12 of the corresponding oxime ether/ketone13–14 pairs 4/5 and 6/7 in low yields (Scheme 1). Trioxolane 1a was similarly obtained as previously described.15 Trioxolanes 2b and 2c were prepared as shown in Scheme 2. In the presence of catalytic p-toluenesulfonic acid (PTSA), 2b was readily formed by condensation of β-hydroperoxy alcohol 8 with 5. Attempts to perform the corresponding reaction of β-hydroperoxy alcohol 9 with 5 provided only small quantities of trioxane 2c accompanied by a complex mixture

Figure 1. Artemisinin and OZ277.
of decomposition products. Characterization of the reaction products indicated that β-hydroperoxy alcohol 9 underwent acid-catalyzed O–O bond (pathway I) and C–O bond (pathway II) heterolysis followed by rearrangement. Formation of ketone 7 (pathway I) can be rationalized by a Grob-type fragmentation or by migration of the hydroxymethyl group. This contrasts with the phenyl migration seen in the acid-catalyzed decomposition of cumene hydroperoxide to form phenol and acetone. Aldehyde 11 may arise from the formation and rearrangement of a relatively stable benzylic tertiary carbocation in the transition state for fragmentation, with the co-generated hydrogen peroxide producing lactone 12 via an acid-catalyzed Baeyer–Villiger oxidation of adamantalone. Thus, acid-catalyzed heterolytic peroxide bond fragmentation (pathway II) was the dominant reaction course for 9. Therefore, we tried protecting the hydroperoxide functional group of 9 to suppress the fragmentation. Treatment of 9 with N,O-bis-(trimethylsilyl)acetamide (BSA) in CH$_2$Cl$_2$ gave the corresponding bis(trimethylsilylated) product 10 in high yield. Subsequent condensation of 10 with 5 in the presence of 10–20% CSA in CH$_2$Cl$_2$ afforded the desired 2c (Scheme 2). Trioxane 2a was prepared as previously described.

Trioxepanes 3a and 3b were prepared via parallel routes (Scheme 3) beginning with alcohol 13 and cinnamyl alcohol 14. The corresponding acetates (15, 16) underwent successive Co-mediated dioxygenation and chemoselective reduction to furnish triethylsilyl peroxyalcohols 17 and 18. The peroxyalcohols underwent HF-mediated condensation with ketone 5 to produce 3a and 3b.

In vitro and in vivo antimalarial activities (Table 1) were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of Plasmodium falciparum and Plasmodium berghei-infected mice. Several observations arise from the antimalarial data. First, trioxolane 1b was more than two or three orders of magnitude less potent than trioxolanes 1a and 1c. Because it has a α-H atom, 1b may lack the necessary chemical
Table 1. Activity of 1–3 against P. falciparum in vitro and P. berghei in vivo

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (ng/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Activity&lt;sup&gt;b&lt;/sup&gt; (%) po</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1</td>
<td>NF54</td>
</tr>
<tr>
<td>NONE</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.97</td>
<td>1.4</td>
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<tr>
<td>1b</td>
<td>400</td>
<td>&gt;1000</td>
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<tr>
<td>1c</td>
<td>2.9</td>
<td>3.4</td>
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<tr>
<td>2a&lt;sup&gt;d&lt;/sup&gt;</td>
<td>54</td>
<td>44</td>
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<td>2b</td>
<td>9.1</td>
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<td>2c</td>
<td>19</td>
<td>22</td>
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<tr>
<td>3a&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>3b&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt;1000</td>
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<tr>
<td>ART&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.6</td>
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<sup>a</sup> Mean from n = 2-3 against chloroquine-resistant (K1) and chloroquine-sensitive (NF54) strains of P. falciparum.

<sup>b</sup> Groups of three P. berghei-infected NMRI mice were treated orally one day post-infection with trioxolanes (100 mg/kg) dissolved or suspended in 5% ethanol and 7% Tween 80. Antimalarial activity was measured by percent reduction in parasitemia on day 3 post-infection. Individual measurements generally differed by less than 10%.

<sup>c</sup> Data from Dong et al. 15

<sup>d</sup> Data from Tang et al. 20

<sup>e</sup> Not determined

<sup>f</sup> ART = artemisinin

stability<sup>24</sup> required for antimalarial activity. This contrasts with the good antimalarial activity of the corresponding and presumably more chemically stable trioxane 2b. Trioxanes 2a and 2c were less potent than the corresponding trioxolanes 1a and 1c; neither 2a nor 2c had any activity in vivo. The less accessible peroxide bond in the trioxane vs trioxolane heterocycle<sup>20</sup> may explain why trioxanes 2a and 2c are less active than their trioxolane chemical cousins 1a and 1c. Similarly, the complete lack of activity of trioxepanes 3a and 3b may arise from peroxide bonds that are too sterically hindered (vide infra). These data suggest that antimalarial activity for these spirodamantryl trioxolanes, trioxanes, and trioxepanes depends on a finely tuned balance between peroxide bond shielding and accessibility. To provide mechanistic insight into this hypothesis, we examined the reactivity of several of these compounds with iron(II) in the form of FeSO₄ and heme.

The reactivity of 1–3 with inorganic ferrous iron was determined using previously established standardized conditions<sup>23</sup>. In these experiments, pseudo-first order reaction rate constants for 1b, 2a, 2b, 3a, and 3b (0.03 mM) with FeSO₄ (3 mM) in 50% acetonitrile/water at 37 °C under an argon atmosphere were determined. These data (n = 3) were corrected for non-specific degradation in iron-free controls. Under these experimental conditions, the previously determined pseudo-first order rate constants (k) for 1a and artemisinin were 0.41 ± 0.02 h<sup>−1</sup> and 0.054 ± 0.006 h<sup>−1</sup><sup>25</sup>. The weakly active 1b reacted considerably more rapidly (k = 1.77 ± 0.06 h<sup>−1</sup>) with iron(II) than did 1a confirming our previous observations<sup>26–27</sup> that chemically reactive peroxides do not necessarily possess high antimalarial activities. Trioxane 2b reacted with iron(II) at a rate (k = 0.12 ± 0.03 h<sup>−1</sup>) between that of 1a and artemisinin, whereas trioxepane 3b reacted much more slowly (k = 0.021 ± 0.006 h<sup>−1</sup>), and neither 2a nor 3a underwent statistically significant degradation over the 24 h time course studied (k = 0.011 ± 0.013 h<sup>−1</sup> and 0.017 ± 0.012 h<sup>−1</sup>, respectively).

As has been previously demonstrated for 1a versus 2a<sup>20</sup>, the difference in iron-mediated reactivity between 1a and 3a can be explained by considering peroxide bond accessibility of the more reactive equatorial peroxide bond conformers<sup>28</sup> as determined by molecular modeling (Spartan’08, Wavefunction, Inc.). As shown in Figure 3, the peroxide bond LUMO in 1a is easily accessible from the cyclohexane side, whereas the peroxide bond LUMO in 3a is not accessible from either direction, consistent with the low reactivity of 3a with iron(II). The greater steric crowding in 3a is due to the methylene groups of the trioxepane ring decreasing the angle between the adamantane and cyclohexane rings. This lack of iron-mediated reactivity for trioxepane 3a is consistent with its lack of antimalarial activity; similar arguments have been put forth to explain the low antimalarial activity of other sterically congested 1,2,4-trioxepanes<sup>29</sup>

We have previously shown<sup>30</sup> a good correlation between in vitro antimalarial activity and heme alkylation efficiency for a series of 22 trioxolanes. We measured the efficiency of heme alkylation for these trioxolanes, trioxanes and trioxepanes using these same experimental conditions<sup>20</sup>. Significant heme alkylation was observed within 30 s for the active 1c (67 ± 2%) and moderately active 2b (55 ± 4%), and to a slightly lesser extent for the less active 1b (50 ± 1%) and 2a (45 ± 1%); however the extent of heme alkylation by these compounds was far lower than previously reported for the highly active trioxolanes 1a (86 ± 0.2%) and OZ277 (83 ± 2%).<sup>31</sup> Trioxolanes 3a and 3b mediated very little heme alkylation (25 ± 1% and 1 ± 0.4%), in agreement with their lack of antimalarial activity. The investigation of heme alkylation appears to give a more accurate prediction of antimalarial activity than investigation of iron-mediated reaction rates, suggesting that antimalarial activity is not merely dependant on peroxide bond cleavage, but also on the ability of reactive intermediates to alkylate heme or other proximal targets. This further supports a likely role for heme alkylation in the antimalarial mechanism of action of peroxide antimalarials<sup>6</sup>.

Acknowledgments — We thank Josefina Santo Tomas and Christopher Snyder for assistance in performing the in vivo antimalarial assays. This investigation received financial support from Medicines for Malaria Venture (MMV).

References & notes

Peroxide bond iron(II) reactivity, heme alkylation efficiency, and antimalarial activity

10. Adamantane-2-spiro-3′-5′-phenyl-1′,2′,4′-trioxolane (1b): 1H NMR (500 MHz, CDCl₃) δ 1.70–1.93 (m, 3H), 2.03–2.16 (m, 4H), 2.22–2.26 (m, 1H), 6.09 (s, 1H), 7.36–7.46 (m, 3H), 7.50–7.56 (m, 2H). 13C NMR (125.7 MHz, CDCl₃) δ 26.5, 26.8, 34.2, 34.6, 34.9, 35.7, 35.8, 36.78, 36.8, 103.7, 113.0, 127.9, 128.5, 130.3, 132.8. Anal. Calcd for C₂₇H₂₇O₃: C, 75.50; H, 7.40. Found: C, 74.94; H, 7.19. Adamantane-2-spiro-3′-5′-methyl-5′-phenyl-1′,2′,4′-trioxolane (1a): mp 70–72 °C. 1H NMR (500 MHz, CDCl₃) δ 1.72 (s, 3H), 1.56–2.20 (m, 14H), 7.28–7.38 (m, 3H), 7.50–7.56 (m, 2H). 13C NMR (125.7 MHz, CDCl₃) δ 25.9, 26.5, 26.9, 34.5, 34.7, 34.78, 35.3, 35.4, 36.6, 36.8, 108.7, 112.9, 125.2, 128.06, 128.11, 142.6. Anal. Calcd for C₂₇H₂₇O₃: C, 75.50; H, 7.74. Found: C, 75.58; H, 7.63. Adamantane-2-spiro-3′-5′-phenyl-1′,2′,4′-trioxane (1c): mp 70–72 °C. 1H NMR (500 MHz, CDCl₃) δ 1.89–2.14 (m, 6H), 2.22–2.42 (m, 2H), 3.85 (dt, J = 12.2, 3.4, 1H), 5.14 (dd, J = 11.5, 3.5, 1H), 7.29–7.40 (m, 5H). 13C NMR (125.7 MHz, CDCl₃) δ 27.16, 27.2, 33.1, 33.6, 33.9, 34.1, 34.7, 35.0, 35.7, 39.4, 60.0, 76.6, 81.6, 108.6, 127.3, 128.4, 128.5, 138.5, 196.0. HRMS-FAB calcd for C₂₇H₂₇O₃ (MH⁺): 301.1804; found 301.1809.

28. The equatorial peroxide conformer provides greater access to the peroxide bond from the cyclohexane side. For 1a and 1a, the energy difference between the two chair conformers (ΔE = Eeq – Eeq) is 0.280 and 1.71 kcal/mol, respectively. 29. R. Amewu, A. V. Stachulski, N. G. Berry, S. A. Ward, J. Davies, G. Labat, J.-F. Rossignol, and P. M. O'Neill, *Bioorg. Med. Chem. Lett.* 16 (2006), p. 6124.