

## Malaria-Infected Mice Live until at Least Day 30 after a New Monomeric Trioxane Combined with Mefloquine Are Administered Together in a Single Low Oral Dose<sup>†</sup>

Lauren E. Woodard,<sup>‡</sup> Wonsuk Chang,<sup>‡</sup> Xiaochun Chen,<sup>§</sup> Jun O. Liu,<sup>§</sup> Theresa A. Shapiro,<sup>||,⊥</sup> and Gary H. Posner<sup>\*,‡,⊥</sup>

<sup>‡</sup>Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218-2685, <sup>§</sup>Department of Pharmacology and Oncology, School of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205,

<sup>||</sup>Division of Clinical Pharmacology, Department of Medicine, School of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205, and <sup>⊥</sup>The Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Baltimore, Maryland 21205

Received May 7, 2009

In only five simple steps and 48% overall yield from the natural trioxane artemisinin, the thermally and hydrolytically stable trioxane fluoroanilide **4b** has been prepared. Upon one oral dose of only 6.8 mg/kg of monomeric trioxane **4b** combined with 20 mg/kg of mefloquine hydrochloride, all of the malaria-infected mice lived until at least day 30 post infection. Of the five mice in this surviving group, four (80%) were completely cured (no parasites in their blood) and one mouse had 4% blood parasitemia. Importantly, the efficacy of this ACT chemotherapy using monomeric trioxane **4b** plus mefloquine hydrochloride is considerably better than the efficacy under the same conditions using the popular trioxane drug artemether plus mefloquine hydrochloride.

### Introduction

Resistance of malaria parasites, especially to chloroquine, is now widespread, seriously compromising the efficacy of this antimalarial drug that has been so widely and successfully used during the past 60 years.<sup>1</sup> Today, as recommended by the World Health Organization (WHO)<sup>2</sup> and as adopted by most countries where malaria is endemic, artemisinin (**1**) combination therapy (ACT<sup>a</sup>) is popular.<sup>3–7</sup> Typically, natural trioxane **1** derivatives like artemether (**2b**) or sodium artesunate (**2c**) are used. One leading ACT drug for chemotherapy of people sick with malaria is a fixed 1:6 combination of the trioxane **2b** with the amino-alcohol lumefantrine.<sup>8</sup> Typically, a six-dose adult regimen requires a total of approximately 320 mg of trioxane **2b** and 1920 mg of lumefantrine. A second ACT drug requires a three-dose adult regimen totaling 600 mg of trioxane **2c** and 750 mg of the quinoline antimalarial mefloquine. Patient compliance with adhering to a repeated dose regimen, however, is often a serious problem. Therefore, a single dose oral cure is highly desirable. Toward this goal, we have recently reported a “proof of principle” advance in malaria chemotherapy: a single 144 mg/kg oral dose cure of malaria-infected mice by a new trioxane dimer sulfone carbamate.<sup>9</sup> We have now prepared and tested the *in vivo* antimalarial efficacies of the fluorobenzyl amide trioxane dimer **3**, of the corresponding trioxane monomer fluorobenzyl amide **4a**, and of the trioxane monomer fluoroanilide **4b** (artefanilide). Monomeric trioxane

fluoroanilide **4b** is the most efficacious; using only one single-digit mg/kg oral dose of this new trioxane monomer combined with a 3-fold higher amount of mefloquine hydrochloride prolonged survival of malaria-infected mice until at least day 30. The total amount of fluoroanilide **4b** and mefloquine hydrochloride needed to achieve this single oral dose high efficacy compares favorably with the amounts of the antimalarial trioxane drugs **2b** and **2c** plus amine currently used clinically in repeated oral dose human ACT chemotherapy (Scheme 1).<sup>8</sup>

### Results and Discussion

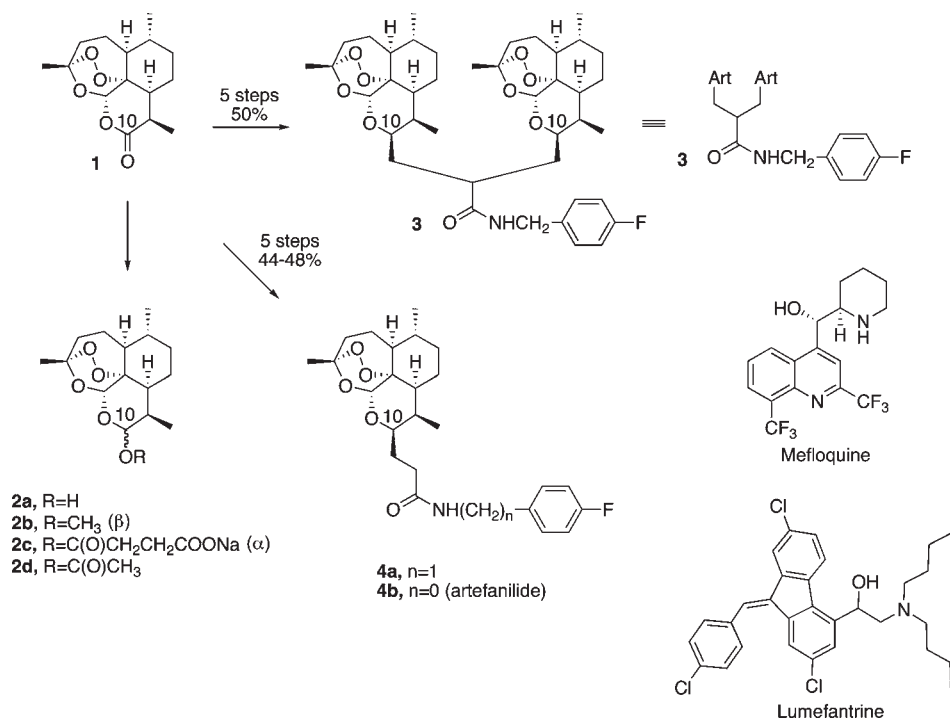
**Chemistry.** Monomeric trioxane dihydroartemisin C-10 acetate (**2d**) has been converted into dimeric trioxane **5** and then into dimer carboxylic acid **6** (Scheme 2).<sup>10</sup> Facile amidation produced trioxane dimer fluorobenzyl amide **3**. Also, C-10 acetate **2d** has been converted into C-10-allyl derivative **7** and then, via hydroboration followed by oxidation, into monomeric trioxane carboxylic acid **8** (Scheme 3).<sup>11</sup> Monomeric trioxane carboxylic acid **8** can be transformed in one step directly into a diverse library of monomeric trioxane amides.<sup>11</sup> For example, one-step amidation of carboxylic acid **8** produced trioxane fluorobenzyl amide **4a**, a monomeric version of dimer fluorobenzyl amide **3** (Scheme 3). In the same way, monomer trioxane fluoroanilide **4b** was prepared directly and in good yield from carboxylic acid **8** (Scheme 3). The overall yield of **4b** is approximately 48% from natural trioxane **1**, and scale up to multigram or even kilogram amounts is expected to be straightforward. Fluoroanilide **4b** is stable as a solid in the absence of solvent for at least 7 days at 60 °C and for at least 1 day at 70 °C. Because amides **3**, **4a**, and **4b** are also C-10 nonacetal trioxanes, they are all more hydrolytically stable than the C-10 acetal trioxane drugs **2b** and **2c**.

<sup>†</sup> In honor of the 100th anniversary of the Division of Medicinal Chemistry

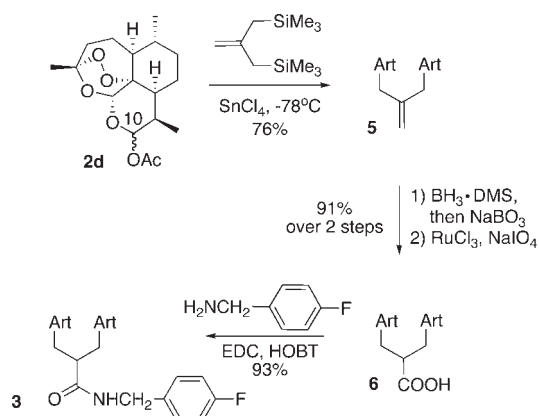
\*To whom correspondence should be addressed. Phone: 410-516-4670. Fax: 410-516-8420. E-mail: ghp@jhu.edu.

<sup>a</sup> Abbreviations: ACT, artemisinin combination therapy; DMSO, dimethyl sulfoxide; DMS, dimethyl sulfide; EDC, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole.

## Scheme 1



## Scheme 2

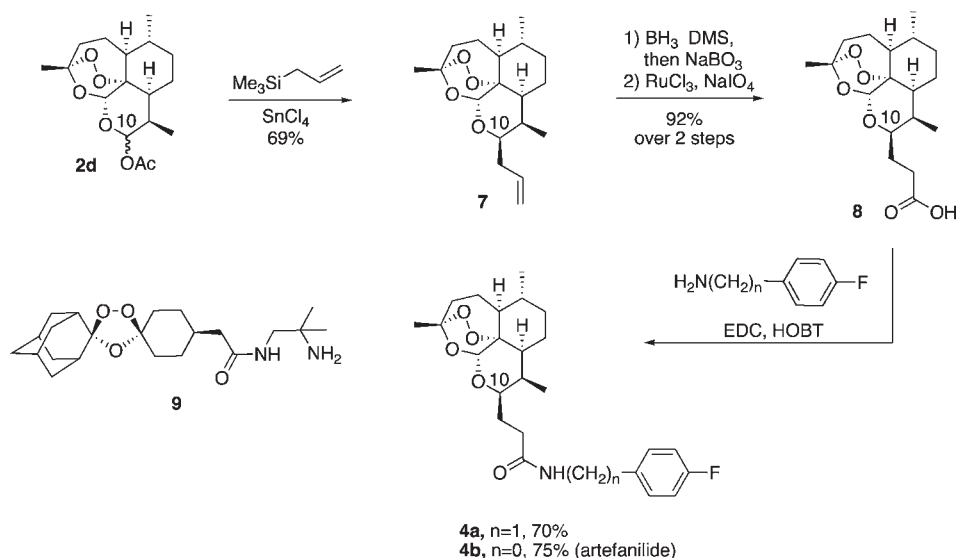


**Biology.** Each trioxane **2b**, **3**, **4a**, and **4b** (0.90 mg) was dissolved in 0.11 mL of 7:3 Tween 80:ethanol and then diluted with 1.10 mL of water for oral administration to 5-week old C57BL/6J male mice (from the Jackson Laboratory) weighing about 22 g that were infected intraperitoneally on day 0 with the *Plasmodium berghei*, ANKA strain ( $2 \times 10^7$  parasitized erythrocytes).<sup>12</sup> Each of five mice in a group was treated orally 24 h postinfection with a single dose of 0.20 mL (0.20 mL/1.21 mL  $\times$  0.9 mg = 0.15 mg) of diluted compound solution, corresponding to a dose of 6.8 mg/kg, combined with 20 mg/kg of mefloquine hydrochloride. Determining blood parasitemia levels as well as monitoring the duration of animal survival compared to survival time of animals receiving no drug are both widely accepted as measures of a drug's efficacy in antimalarial drug development. Three days after infection, an average of 16% blood parasitemia was observed in the control (no drug) group. Animals receiving no drug died on days 6–7 post infection. A widely accepted yardstick of cure (i.e., 100% efficacy) is survival of animals to day 30 post infection, with no detectable malaria parasites in the animal's

blood at that time. Average survival results are summarized in Table 1, including single oral doses of 13 mg/kg of trioxane combined with 13 mg/kg of mefloquine hydrochloride. The clinically used monomeric water-soluble trioxane drug **2c** and the synthetic trioxolane peroxide drug development candidate OZ277 (**9**) maleate are included as monotherapy reference compounds.

It is clear from the data in Table 1 that all three of the new trioxane fluorinated amides **3**, **4a**, and **4b** plus mefloquine hydrochloride prolonged average survival time much more effectively than monotherapy using trioxolane **9**, which is in phase II clinical trials.<sup>13</sup> A nonfluorinated version of dimeric trioxane fluoroanilide **3** was much less antimalarially efficacious than fluorobenzyl amide **3** (data not shown). It is also apparent from the data in Table 1 that the monomeric trioxane fluoroanilide **4b**, at a single oral dose of only 6.8 mg/kg plus 20 mg/kg of mefloquine hydrochloride, was the most efficacious at prolonging survival. Of the five mice in this 30-day surviving group, four (80%) were completely cured (no parasites in their blood) on day 30 post infection and one mouse had 4% blood parasitemia. This trioxane fluoroanilide **4b** caused a 99.0% suppression of parasitemia on day 3 post infection. A single oral dose of 13 mg/kg of trioxane fluoroanilide **4b** combined with 13 mg/kg of mefloquine hydrochloride also prolonged survival of all of the mice until day 30, with four mice cured and one mouse having 2% blood parasitemia. Reinfection of the four cured mice on day 32 after the original infection caused an extended survival time, approximately four days longer than the average survival time of a newly infected control group; the mechanism(s) for this protective effect of trioxane fluoroanilide **4b** against malaria reinfection is not clear at this time. In monotherapy control experiments, a single high oral dose (72 mg/kg) of the trioxane fluoroanilide **4b** prolonged average survival until day 24.4, and a single oral dose (20 mg/kg) of mefloquine hydrochloride alone prolonged average survival to day 17.6. In an ACT control experiment,

## Scheme 3

**Table 1.** Antimalarial Efficacy Using a Single Oral Dose of Trioxane Combined with Mefloquine Hydrochloride in *Plasmodium berghei*-Infected Mice

Trioxane	Oral Dose		Average survival (days) after infection	% Suppression of parasitemia (on day 3 post infection)
	Trioxane (mg/kg)	Mefloquine Hydrochloride (mg/kg)		
<b>2b</b>	6,8	20	19.8 (16,17,21,22,23) <sup>a</sup>	99.8
<b>2b</b>	13	13	19.6 (16,17,21,22,22) <sup>a</sup>	99.7
<b>3</b>	6.8	20	27.4 (22,25,30,30,30) <sup>a</sup>	99.5
<b>3</b>	13	13	24.6 (17,21,25,30,30) <sup>a</sup>	99.9
<b>4a</b>	6.8	20	27.8 (22,27,30,30,30) <sup>a</sup>	99.9
<b>4a</b>	13	13	26.4 (21,23,26,30,30) <sup>a</sup>	99.6
<b>4b</b>	6.8	20	30	99.0
<b>4b</b>	13	13	30	99.6
<b>4b</b>	72	0	24.4 (21,23,24,27,27) <sup>a</sup>	99.0
<b>Controls</b>				
vehicle (no drug)	0	0	6.4 (6,6,6,7,7) <sup>a</sup>	0
<b>2c</b>	30	0	7.6 <sup>b</sup>	- <sup>c</sup>
<b>9</b>	30	0	10.7 <sup>b</sup>	99.95 <sup>b</sup>
Mefloquine	0	20	17.6 (16,16,17,19,20) <sup>a</sup>	99.6

<sup>a</sup> Actual mouse survival until day. <sup>b</sup> Data from Supporting Information in ref 13 using a single oral dose of 30 mg/kg. <sup>c</sup> Dash indicates "not measured".

6.8 mg/kg of the popular trioxane drug **2b** combined with 20 mg/kg of mefloquine hydrochloride prolonged average survival to only day 19.8 (Table 1). Neither overt toxicity nor behavioral change attributable to trioxane drug administration was observed in any of the malaria-infected animals cured by trioxane fluoroanilide **4b** plus mefloquine hydrochloride combination. The water-soluble monomeric trioxane antimalarial drug **2c** and the trioxolane antimalarial drug candidate **9**, although able to lower parasitemia levels considerably by day 3 post infection, were not efficacious in prolonging the mouse average survival time beyond day 11 when used as monotherapy at a dose of 30 mg/kg.

**Conclusions**

In conclusion, a single-digit oral dose of any one of the three new trioxane fluorinated amides **3**, **4a**, and **4b** combined with mefloquine hydrochloride is considerably more antimalarially efficacious than the popular ACT trioxane drug **2b** combined with mefloquine hydrochloride.<sup>14,15</sup> Monomer trioxane fluoroanilide **4b** stands out as being the most powerful antimalarial in this series of semisynthetic trioxane fluorophenyl amides. Preclinical drug development is continuing on trioxane lead optimization and on use of other amine combination partners.

## Experimental Section

High-pressure liquid chromatography (HPLC) was performed on a Rainin HPLX system equipped with two 25 mL pump heads and a Rainin Dynamax UV-C dual-beam variable wavelength detector set at 254 using a Phenomenex Luna 5  $\mu$  C18 250 mm  $\times$  10 mm column. The purity of analogs **3**, **4a**, and **4b** was  $\geq 98\%$  based on HPLC analysis.

**Synthesis of Trioxane Dimer Fluorobenzyl Amide (3).** To a solution of acid **6**<sup>10</sup> (600 mg, 0.967 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) were added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (222 mg, 1.16 mmol) and 1-hydroxybenzotriazole (157 mg, 1.16 mmol) and it was stirred for 1 h at rt. To the reaction were added 4-fluorobenzylamine (0.33 mL, 2.9 mmol), and the solution was stirred for 5 h. It was quenched with water (3 mL). Layers were separated and the aqueous layer was extracted with EtOAc (2  $\times$  4 mL). The combined organic solution was dried (MgSO<sub>4</sub>) and concentrated. The residue was purified by flash column chromatography (elution with EtOAc:hexanes = 1:3) to provide **3** (651 mg, 93%) as a white solid:  $[\alpha]_{\text{D}}^{24} = +82.1$  ( $c = 1.55$ , CHCl<sub>3</sub>); mp = 110 °C. IR (thin film) 3312, 2939, 1669, 1510, 1377, 1221, 1052, 1012, 735 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.32 (m, 2H), 6.97 (m, 2H), 6.23 (t,  $J = 5.6$  Hz, 1H), 5.27 (s, 1H), 5.20 (s, 1H), 4.41 (s, 1H), 4.39 (s, 1H), 4.09 (m, 2H), 2.76 (dq,  $J = 13.2, 7.2$  Hz, 1H), 2.66 (dq,  $J = 13.6, 6.4$  Hz, 1H), 2.54 (octet,  $J = 4.0$  Hz, 1H), 2.31 (m, 2H), 2.18 (m, 1H), 2.01–1.95 (m, 3H), 1.92–1.18 (m, 24H including s at 1.35 and 1.26), 0.98–0.79 (m, 14H including d at 0.95 with  $J = 5.6$  Hz, 0.93 with  $J = 6.0$  Hz, 0.85 with  $J = 7.6$  Hz, and 0.82 with  $J = 7.2$  Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  175.8, 160.8, 134.3, 129.8, 129.7, 115.3, 115.1, 103.4, 102.9, 100.8, 88.6, 88.4, 81.2, 81.1, 76.4, 73.7, 52.5, 52.4, 44.7, 44.5, 44.3, 43.3, 37.4, 37.2, 36.5, 34.5, 33.3, 32.9, 30.2, 29.9, 26.2, 26.0, 24.9, 24.8, 24.6, 24.5, 20.2, 13.5, 13.0. <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>)  $\delta$  -115.7. HRMS (FAB) calculated for C<sub>41</sub>H<sub>59</sub>FNO<sub>9</sub> [(M + H)<sup>+</sup>] 728.4174, found 728.4177.

**Synthesis of Trioxane Monomer Fluorobenzyl Amide (4a).** Into a flame-dried 5 mL RBF was charged acid **8**<sup>11</sup> (75 mg, 0.22 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (67 mg, 0.35 mmol), and 1-hydroxybenzotriazole (35 mg, 0.26 mmol). Dichloromethane (2.5 mL) was then added, and the mixture was stirred for an hour, at which time 4-fluorobenzylamine (95  $\mu$ L, 0.84 mmol) was added by syringe. The reaction was allowed to stir at room temperature for 3 h. It was then quenched with 1 N HCl, extracted with dichloromethane (3  $\times$  5 mL), washed with aqueous NaHCO<sub>3</sub> and brine, dried over magnesium sulfate, and evaporated. The crude product was purified by preparative thin layer chromatography (silica gel, 100% diethyl ether) to afford **4a** as an amorphous, white solid (69 mg, 0.15 mmol, 70%). IR (thin film) 3321, 2947, 2875, 1648, 1546, 1510, 1453, 1378, 1223, 1127, 1095, 1052, 1011, 940, 879, 822, 756 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.25 (m, 2H), 7.00 (m, 2H), 6.08 (br. s, 1H), 5.27 (s, 1H), 4.40 (m, 2H), 4.09 (m, 1H), 2.69 (m, 1H), 2.46 (m, 1H), 2.33 (m, 2H), 2.04–1.77 (m, 5H), 1.64–1.55 (m, 2H), 1.48–1.20 (m, 7H), including singlet at 1.36), 0.99–0.92 (m, 4H), 0.85 (d, 3H,  $J = 6.0$  Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  172.97, 163.40, 160.96, 134.27, 129.54, 115.41, 103.28, 88.91, 81.14, 75.75, 52.44, 44.45, 43.01, 37.43, 36.58, 34.64, 34.48, 30.22, 26.11, 25.15, 24.89, 24.68, 20.12, 13.00;  $[\alpha]_{\text{D}}^{22} = +72$  ( $c = 0.97$ , CHCl<sub>3</sub>). HRMS (FAB)  $m/z$  calcd for C<sub>25</sub>H<sub>34</sub>FNO<sub>5</sub>Na (M + Na)<sup>+</sup> 470.2313, found 470.2300.

**Synthesis of Trioxane Monomer Fluoroanilide (4b).** Into a flame-dried 5 mL RBF was charged carboxylic acid **8**<sup>11</sup> (55 mg, 0.16 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (50 mg, 0.26 mmol), and 1-hydroxybenzotriazole (30 mg, 0.19 mmol). Dichloromethane (2.5 mL) was then added and the mixture was stirred for an hour, at which time 4-fluoroaniline (60  $\mu$ L, 0.61 mmol) was added by syringe. The reaction was allowed to stir at room temperature for 3 h. It was

then quenched with 1 N HCl, extracted with dichloromethane (3  $\times$  5 mL), washed with aqueous NaHCO<sub>3</sub> and brine, dried over magnesium sulfate, and evaporated. The crude product was purified by flash column chromatography (silica gel, 30% ethyl acetate/hexanes) to afford **4b** (artefanilide) as an amorphous, white solid (51 mg, 0.12 mmol, 75%). IR (thin film) 3313, 2939, 2874, 1663, 1614, 1543, 1509, 1451, 1406, 1377, 1212, 1124, 1091, 1055, 1012, 876, 835, 754 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.07 (br. s, 1H), 7.50 (m, 2H), 6.96 (m, 2H), 5.34 (s, 1H), 4.14 (m, 1H), 2.73 (m, 1H), 2.60 (m, 1H), 2.46 (m, 1H), 2.31 (m, 1H), 2.02–1.78 (m, 5H), 1.65–1.55 (m, 2H), 1.47–1.20 (m, 7H), including singlet at 1.35), 0.97–0.93 (m, 4H), 0.87 (d, 3H,  $J = 7.6$  Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.33, 157.89, 134.17, 121.63, 115.33, 103.42, 88.79, 81.08, 76.12, 52.30, 44.31, 37.34, 36.42, 35.62, 34.32, 30.85, 30.13, 25.98, 24.82, 24.55, 20.09, 13.04;  $[\alpha]_{\text{D}}^{22} = +60$  ( $c = 0.47$ , CHCl<sub>3</sub>). HRMS (FAB)  $m/z$  calcd for C<sub>24</sub>H<sub>33</sub>FNO<sub>5</sub> (M + H)<sup>+</sup> 434.2343, found 434.2335.

**Acknowledgment.** We thank Nirbhay Kumar (JHU) for a gift of the *P. berghei* malaria parasites, the NIH (AI 34885 to G.H.P.), the Johns Hopkins Malaria Research Institute, and the Bloomberg Family Foundation for financial support (to G.H.P. and J.O.L.).

**Supporting Information Available:** <sup>1</sup>H, <sup>13</sup>C NMR spectra for all of the new trioxanes **3**, **4a**, and **4b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Olliaro, P. L.; Boland, P. B. Clinical public health implications of antimalarial drug resistance. In *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery*; Rosenthal, P. J., Ed.; Humana Press: Totowa, NJ, 2001; pp 65–83.
- Guidelines for the Treatment of Malaria; World Health Organization: Geneva, 2006.
- Ashley, E. A.; White, N. J. Artemisinin-based combinations. *Curr. Opin. Infect. Dis.* **2005**, *18*, 531–536.
- (a) Adjuk, M.; Babiker, A.; Garner, P.; Olliaro, P.; Taylor, W.; White, N. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* **2004**, *363*, 9–17. (b) Guthmann, J.-P.; Cohuet, S.; Rigutto, C.; Fortes, F.; Saraiva, N.; Kiguli, J.; Kyomuhendo, J.; Francis, M.; Noel, F.; Mulemba, M. Balkan, S. High efficacy of two artemisinin-based combinations (artesunate + amodiaquine and artemether + lumefantrine) in Caala, Central Angola. *Am. J. Trop. Med. Hyg.* **2006**, *75*, 143–145.
- Myint, H. Y.; Ashley, E. A.; Day, N. P. J.; Nosten, F.; White, N. J. Efficacy and safety of dihydroartemisinin–piperaquine. *Trans. R. Soc. Trop. Med. Hyg.* **2007**, *101*, 858–866.
- Sirima, S. B.; Tiono, A. B.; Gansane, A.; Diarra, A.; Ouedraogo, A.; Konate, A. T.; Kiechel, J. R.; Morgan, C. C.; Olliaro, P. L.; Taylor, W. R. *J. Malar. J.* **2009**, *8*, 48.
- de Pilla Varotti, F.; Botelho, A. C. C.; Andrade, A. A.; de Paula, R. C.; Fagundes, E. M. S.; Valverde, A.; Mayer, L. M. U.; Mendonca, J. S.; de Souza, M. V. N.; Boechat, N.; Krettli, A. U. Synthesis, antimalarial activity, and intracellular targets of MEFAS, a new hybrid compound derived from mefloquine and artesunate. *Antimicrob. Agents Chemother.* **2008**, *52*, 3868–3874.
- Sagara, I.; Diallo, A. D.; Kone, M.; Coulibaly, M.; Diawara, S. I.; Guindo, O.; Maiga, H.; Niambele, M. B.; Sissoko, M.; Dicko, A.; Djimde, A.; Doumbo, O. K. A randomized trial of artesunate–mefloquine versus artemether–lumefantrine for treatment of uncomplicated *Plasmodium falciparum* malaria in Mali. *Am. J. Trop. Med. Hyg.* **2008**, *79*, 655–661.
- Rosenthal, A. S.; Chen, X.; Liu, J. O.; West, D. C.; Hergenrother, P. J.; Shapiro, T. A.; Posner, G. H. Malaria-infected mice are cured by a single oral dose of new dimeric trioxane sulfones which are also selectively and powerfully cytotoxic to cancer cells. *J. Med. Chem.* **2009**, *52*, 1198–1203.
- Posner, G. H.; Paik, I.-H.; Sur, S.; McRiner, A. J.; Borstnik, K.; Xie, S.; Shapiro, T. A. Orally active, antimalarial, anticancer, artemisinin-derived trioxane dimers with high stability and efficacy. *J. Med. Chem.* **2003**, *46*, 1060–1065.

- (11) Jung, M.; Lee, S.; Ham, J.; Lee, K.; Kim, H.; Kim, S. K. Antitumor activity of novel deoxoartesimisinin monomers, dimers, and trimer. *J. Med. Chem.* **2003**, *46*, 987–994.
- (12) Chen, X.; Chong, C. R.; Shi, I.; Yoshimoto, T.; Sullivan, D. J., Jr.; Lin, J. O. Inhibitors of *Plasmodium falciparum* methionine aminopeptidase 1b possess antimalarial activity. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 14548–14553.
- (13) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, F. C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Santo, T. J.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N. Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature* **2004**, *430*, 900–904.
- (14) Sagara, I.; Rulisa, S.; Mbacham, W.; Adam, I.; Sissoko, K.; Maiga, H.; Traore, O. B.; Dara, N.; Dicko, Y. T.; Dicko, A.; Djimde, A.; Jansen, F. H.; Doumbo, O. K. Efficacy and safety of a fixed dose artesunate–sulphamethoxypyrazine–pyrimethamine compared to artemether–lumefantrine for the treatment of uncomplicated *falciparum* malaria across Africa: a randomized multicentre trial. *Malar. J.* **2009**, *8*, 63.
- (15) Gautam, A.; Ahmed, T.; Batra, V.; Paliwal, J. Pharmacokinetics and Pharmacodynamics of Endoperoxide Antimalarials. *Curr. Drug. Metab.* **2009**, *10*, 289–306.