Antimalarial Simplified 3-Aryltrioxanes: Synthesis and Preclinical Efficacy/Toxicity Testing in Rodents

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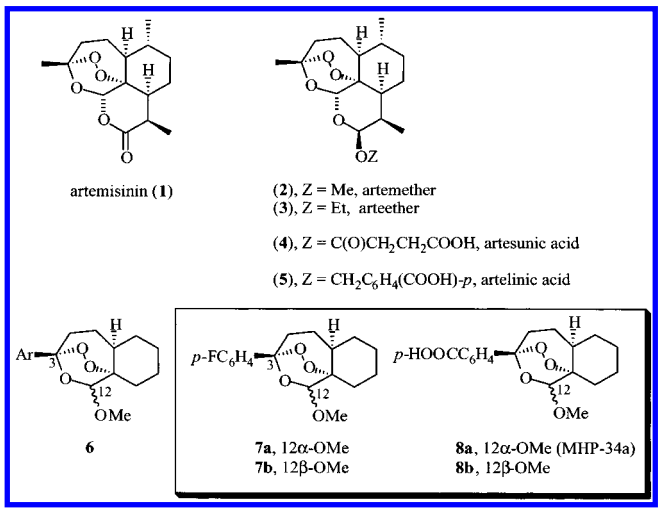
A streamlined five-step chemical synthesis of rationally designed, simplified 3-aryltrioxane **8a** is described. A noteworthy feature of this synthetic scheme is use of air rather than expensive molecular oxygen as the source of the pharmacologically critical peroxide unit in trioxane **8a**. This simplified acetal trioxane carboxylic acid **8a** is thermally stable, and it is hydrolytically stable in water even at 40 °C and pH 7.4 for at least 7 days. Preclinical evaluation of this water-soluble synthetic trioxane **8a** in rodents shows it to have at least as good a therapeutic index (efficacy/toxicity) as that of water-soluble semisynthetic trioxane artelinic acid (**5**).

Introduction

Over two billion people worldwide are currently at risk of contracting malaria.[1] Unfortunately, no vaccine is now available for effective protection against malaria, and chemotherapy of this infectious disease is becoming more and more difficult due to the widespread resistance of the *Plasmodium falciparum* malaria parasite to standard quinoline-based antimalarial drugs such as chloroquine and mefloquine.[2] A promising nonalkaloid class of new, potent, and fast-acting antimalarial trioxanes, based on ancient Chinese folk herbal medicine, is now being developed for clinical use; natural trioxane artemisinin (**1**) and especially its semisynthetic derivatives artemether (**2**), arteether (**3**), and artesunic acid (**4**) are currently used drugs for treating individuals who are infected with malaria.[3−10] Semisynthetic water-soluble trioxane artelinic acid (**5**), designed by the U.S. Walter Reed Army Institute of Research to be more stable than artesunic acid (**4**), is currently a leading candidate for antimalarial drug development.[11,12]



On the basis of our detailed understanding at the molecular level of the chemical cascade leading from antimalarial trioxane to various cytotoxic chemical intermediates,[13,14] we have rationally designed and easily synthesized a series of structurally simple 3-aryltrioxanes **6**. Water-insoluble 3-*p*-fluorophenyl trioxane **7a**, described by us previously,[15] has now undergone preclinical toxicity evaluation in rodents. It is safer than the currently used antimalarial drug arteether (**3**). Solubility in water is important for convenient intravenous (iv) administration of any antimalarial trioxane. Water-soluble 3-*p*-carboxyphenyltrioxanes **8**, described here for the first time, are prepared in only five steps from cyclohexanone, including practical use of air (rather than pure O_2)[16] for IR lamp-induced photochemical introduction of the peroxide unit. Because pure 12α-methoxy diastereoisomer **8a** is more easily obtained chromatographically pure than 12*β*-methoxy diastereoisomer **8b**, we have selected the new chemical entity **8a** for detailed study.

Chemistry

Five-step preparation of synthetic trioxanes **8** is outlined in Scheme 1. Highlights of this streamlined synthetic route include the following: (1) use of a styrene carbon−carbon double bond as a masked form of a water-solubilizing carboxyl group; (2) use of readily available and inexpensive air instead of expensive cylinders of purified molecular oxygen for IR lamp-induced formation of styryl trioxane **10**; (3) optimization of the photooxygenation step after trying diverse silyl triflate and tertiary amines; and (4) final oxidative cleavage of the vinyl group in styryl trioxane **10** to form benzoic acid trioxanes **8** without disturbing the trioxane peroxide linkage that is the crucial antimalarial pharmacophore. Noteworthy also is formation of styryl ketone **9** using the corresponding styryllithium organometallic reagent formed in situ via *tert*-butyllithium reaction with commercial *p*-bromostyrene, without anionic polymerization of the styrene system.[17]

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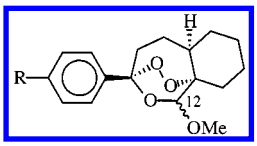
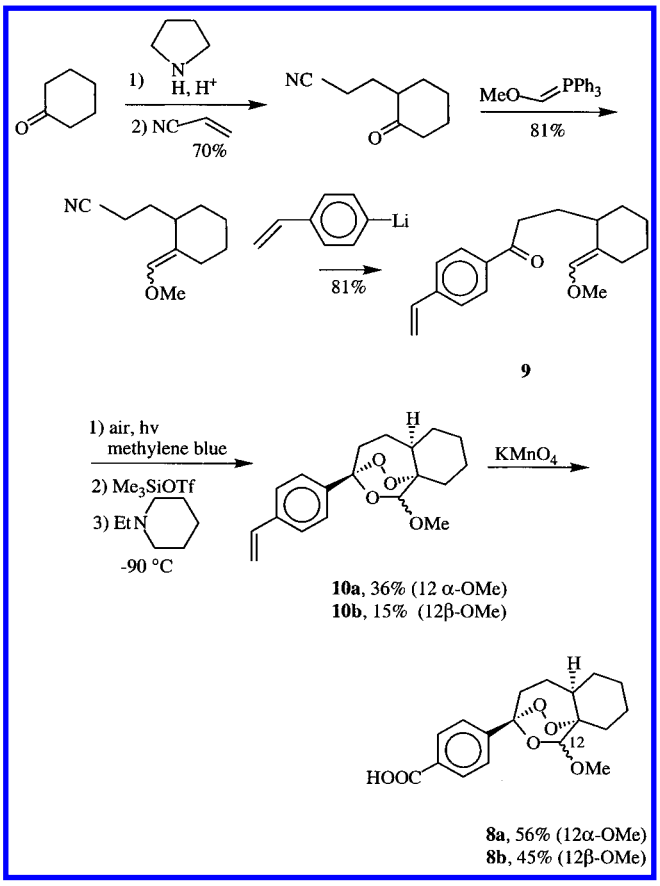
Table 1. Antimalarial Efficacy in Mice Against *P. berghei*



compd	R	ED_{50}, mg/kg[a] (μmol/kg) sc	ED_{50}, mg/kg[a] (μmol/kg) iv	ED_{90}, mg/kg[a] (μmol/kg) sc	ED_{90}, mg/kg[a] (μmol/kg) iv
7a (12α-OMe)	F	4.7 (15.3)	4.0 (13.0)	8.3 (27.0)	8.0 (26.0)
8a (12α-OMe)	HOOC	22.5 (67.4)	19.5 (58.4)	112 (335)	55.0 (165)
8b (12β-OMe)	HOOC	10.5 (31.4)	16.5 (49.4)	19.5 (58.4)	48.0 (144)
arteether (**3**)		0.9 (2.9)	0.46 (1.5)	1.4 (4.5)	
artelinic acid (**5**)		6.6 (15.8)	4.3 (10.3)	14.0 (33.6)	15.5 (37.2)
chloroquine		1.0 (2.0)	5.0 (10.1)		

[a] Four different doses (1, 3, 10, and 30 mg/kg) were administered each day for four days to five mice per dose regimen to establish the ED values indicated here using a previously described protocol.[18]

Scheme 1



Biology

Water-insoluble 3-fluorophenyltrioxane **7a** was evaluated for preclinical efficacy and toxicity in rodents. In mice, efficacy against *P. berghei* malaria parasites was studied using both subcutaneous (sc) and intravenous (iv) modes of administration.[18] Results are summarized in Table 1. Relative to water-insoluble semisynthetic arteether (**3**), synthetic fluorophenyl trioxane **7a** is about 5-fold less efficacious via sc administration and about 8-fold less efficacious via iv administration. In rats, a 14-day iv toxicity study, however, showed synthetic trioxane **7a** to be significantly safer than arteether (**3**). Relative to vehicle controls, arteether (**3**) at 160 μmol/kg/day caused a time-dependent (i) reduction in expected weight gain (Figure 1A); (ii) reduction in red blood cell mass (red cell count, hemoglobin, and hematocrit) and increase in reticulocyte count (Figure 1B and data not shown); and (iii) increase in liver function tests (alanine aminotransferase, aspartate aminotransferase, and total bilirubin; Figure 1C and data not shown). At necropsy, there were neuronal abnormalities in brain tissue. These changes indicate toxicity to circulating red cells, liver cells, and brain cells. In contrast, synthetic trioxane **7a** at the same dose caused a less marked reduction in red cell mass and increase in reticulocytes, with no changes in weight gain, liver function tests, or brain histopathology (Figure 1A−C and data not shown).

Water-soluble 3-carboxyphenyltrioxane **8a** was evaluated also in the same manner, in comparison with water-soluble artelinic acid (**5**). In mice, synthetic trioxane **8a** is about 4-fold less efficacious against *P. berghei* malaria parasites than semisynthetic artelinic acid (**5**) via sc administration and about 5-fold less efficacious via iv administration (Table 1).[18] Synthetic trioxane **8a** is 3−5 times more soluble in water at pH 7.4 than is artelinic acid (**5**). Relative to buffer controls, artelinic acid (**5**) at 26 μmol/kg/day (the limit of solubility) caused a time-dependent (i) reduction in red blood cell mass (red cell count, hemoglobin, and hematocrit) and increase in reticulocyte count (Figure 2B and data not shown) and (ii) increase in alanine aminotransferase (Figure 2C), indicative of red cell and liver toxicity. However, water-soluble **8a**, at a dose 6 times greater than that of artelinic acid, caused no detectable toxicity. Thus, the therapeutic index for water-soluble synthetic trioxane **8a** is at least as good as that of artelinic acid (**5**).

To be a promising antimalarial drug candidate, any new trioxane must have a long shelf life. The thermal stability of synthetic acetal trioxane carboxylic acid **8a** is very good. For example, heating this crystalline trioxane in air at 60 °C for 24 h caused less than 5% decomposition, as judged by ^{1}H NMR spectroscopy. Also, heating a pH 7.4 phosphate-buffered 0.1 N NaCl solution of trioxane **8a** in air at 40 °C for 7 days also caused less than 5% decomposition, as determined by ^{1}H NMR spectroscopy.

In conclusion, synthetic water-soluble trioxane **8a** is a promising antimalarial drug candidate. More direct side-by-side preclinical testing of water-soluble trioxanes **5** and **8a** in larger animals is now appropriate to establish the generality of the physiological observations reported here and to allow a reliable ranking of the

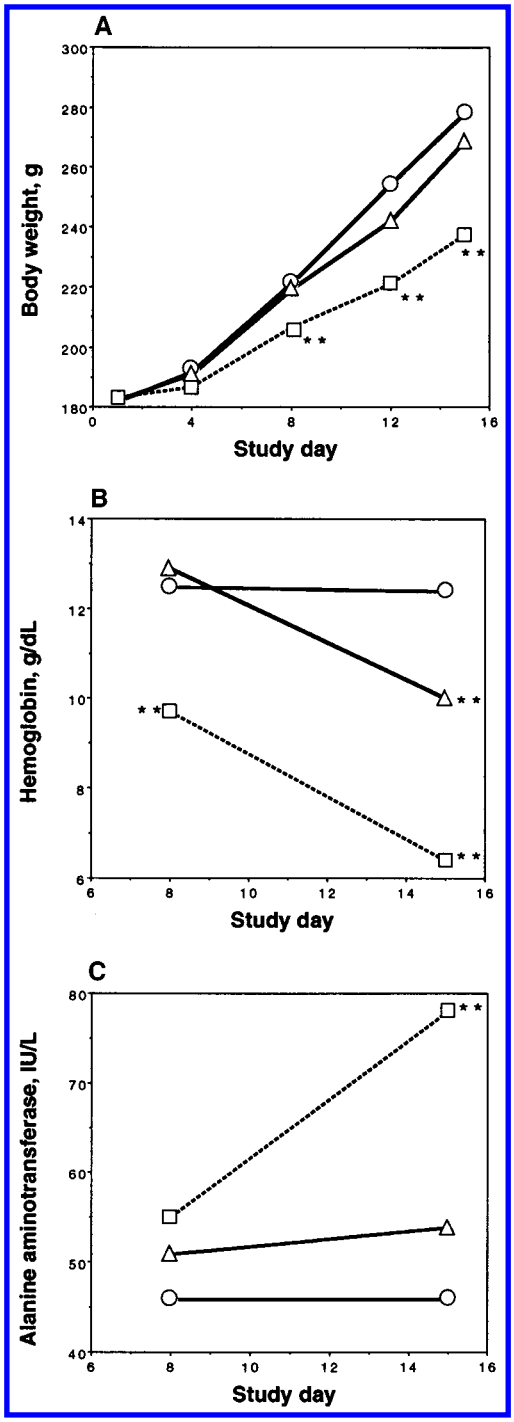


Figure 1. Toxicity study of water-insoluble trioxanes. Compounds were formulated in an emulsion and administered intravenously to rats once daily for 14 days, at 160 μmol/kg/day arteether (open squares); 160 μmol/kg/day **7a** (open triangles); or comparable volume of vehicle (open circles). Body weight (A), hemoglobin (B), and alanine aminotransferase (C) were measured on the indicated days of dosing. Values are means from five animals. Double asterisk, value different from vehicle control at $p < 0.01$.

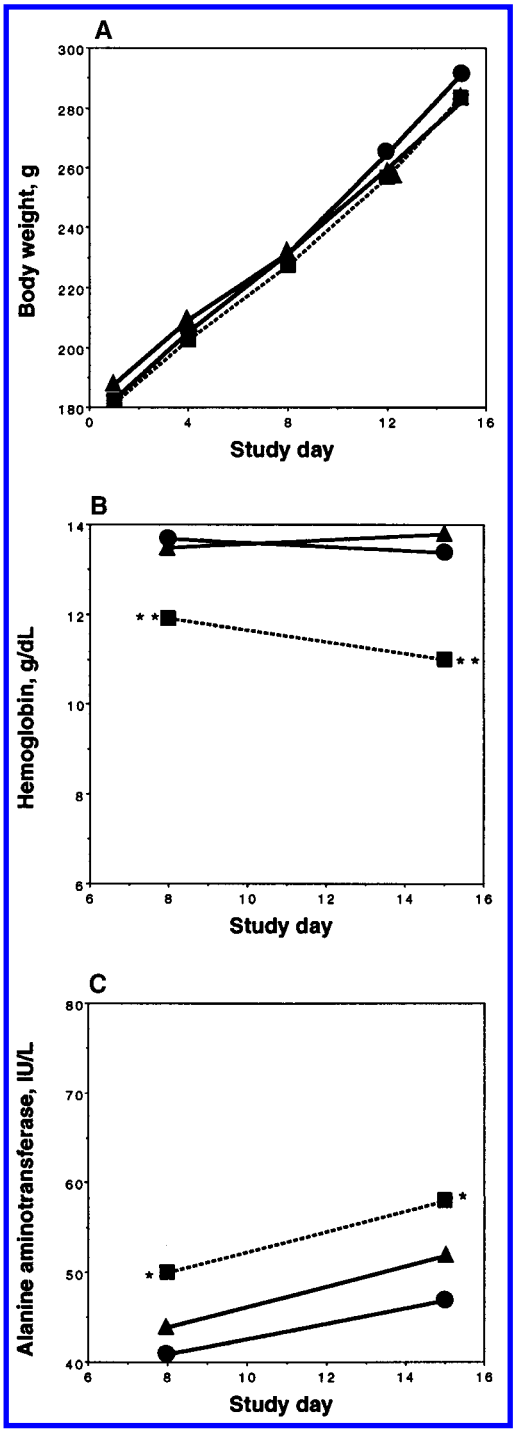


Figure 2. Toxicity study of water-soluble trioxanes. Compounds were dissolved in phosphate-buffered saline and administered intravenously to rats once daily for 14 days, at 26 μmol/kg/day artelinic acid (closed square); 160 μmol/kg/day **8a** (closed triangle); or comparable volume of buffer (closed circles). Body weight (A), hemoglobin (B), and alanine aminotransferase (C) were measured on the indicated days of dosing. Values are means from five animals. Single asterisk, value different from buffer control at $p < 0.05$; double asterisk, $p < 0.01$.

therapeutic potential of these two promising water-soluble antimalarial drug candidates.

Experimental Section

Preparation of 2-(2-Cyanoethyl)-1-(methoxymethylene)cyclohexane. An oven-dried 500 mL one-necked round-bottomed flask was charged with (methoxymethyl)triphenylphosphonium chloride (23.8 g, 69.4 mmol, used as received from Aldrich Chem. Co.) and dry THF (180 mL) and then cooled to −78 °C. To this solution was added *n*-BuLi (1.6 M in hexane, 43.4 mL, 69.4 mmol) over 10 min by syringe. The resultant red ylide solution was then warmed to room temperature, stirred for 3 h, and cooled to −78 °C. To this ylide solution was added 2-cyanoethylcyclohexanone[19] (7.0 g, 46.3 mmol) in dry THF (50 mL) via cannula over 10 min. After

being stirred for 1 h at −78 °C, the reaction mixture was warmed to room temperature slowly, stirred for 10 h at room temperature, and then cooled to 0 °C, quenched with water (50 mL), and diluted with ether (50 mL). The organic layer was separated from aqueous layer and further extracted with ether (70 mL × 2), combined, and washed with saturated NaCl solution (60 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to column chromatography with EtOAc−hexane (1:12) as eluent to give the product (6.7 g, 81%) as yellow oil, having the same spectroscopic properties as those of an authentic sample.[19, 20]

Preparation of *p*-Styryl Ketone 9. To a solution of *p*-styryl bromide (1.1 mL, 8.0 mmol) in ether (30 mL) at −78 °C was added *t*-BuLi (5.4 mL, 1.7 M in pentane, 9.2 mmol) via syringe over 1 min. The resulting dark-red solution was stirred at −78 °C for 15 min. A precooled (−78 °C) solution of the above nitrile (1.1 g, 6.1 mmol) in ether (25 mL) was then added dropwise via cannula during 3 min. The resulting mixture was stirred at −78 °C for 15 min, then the cooling bath was removed, and the reaction mixture was allowed to reach room temperature. At this point TLC analysis indicated full consumption of the starting material. The reaction was quenched with 10 mL of saturated aqueous $NaHCO_3$, poured into a separatory funnel containing 200 mL of ether and 50 mL of water. The organic layer was further washed with 50 mL of saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography with EtOAc−hexane (1:15) as eluent to give the ketone **9** (1.4 g, 81%) as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.45 (m, 2H), 6.73 (dd, J = 17.6, 5.4 Hz, 1H), 5.85 (dm, J = 8.8 Hz, 1H), 5.78 (br s) and 5.72 (s) − 1H total, 5.37 (dt, J_d = 9.6 Hz, J_t = 0.8 Hz) and 5.36 (dt, J_d = 10.8 Hz, J_t = 0.8 Hz) − 1H total, 3.48 (m) and 3.39 (m) − 3H total, 2.88 (m, 2H), 2.27 (dt, J_d = 14.0 Hz, J_t = 4.8 Hz, 1H), 2.00 (m, 3H), 1.43−1.83 (m, 6H), 1.16−1.42 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.2, 200.0, 141.7, 141.5, 140.4, 139.6, 136.4, 136.2, 135.9, 135.8, 128.3, 126.2, 126.1, 119.7, 119.0, 116.4, 116.2, 59.2, 59.0, 38.5. 36.8, 36.7, 33.5, 32.6, 31.6, 28.2, 27.2, 26.4, 26.2, 25.8, 23.0, 22.6, 21.6. IR (neat) 2925, 2852, 1682, 1604, 1448, 1404, 1233, 1123, 989, 915, 841 cm^{-1}. Due to the tendency of styryl systems to polymerize, styryl ketone **9** was used immediately in the next step.

Preparation of *p*-Styryl Trioxane 10 at −90 °C. A 100 mL three-necked round-bottom flask equipped with dispersion bubbler gas inlet, gas outlet, and a septum was charged with *p*-styryl ketone **9** (*E*/*Z* = ∼1/1, 80 mg, 0.28 mmol) in dry CH_2Cl_2 (30 mL), and methylene blue (1.1 mg) was added. To the solution maintained at −90 °C by liquid N_2 in MeOH was bubbled dry air (flow rate = ∼240 mL/min) with a 250W IR lamp (General Electric) at 1 in. distance from the reaction flask. During the reaction, the temperature was maintained between −80 °C and −84 °C. TLC analysis after 15 min showed no starting material, at which time the IR lamp was removed. The air bubbler and outlet also were removed, and the reaction was placed under Ar atmosphere. A precooled (−78 °C) solution of Me_3SiOTf (TMSOTf, 0.08 mL, 0.42 mmol) in CH_2Cl_2 (5 mL) was added slowly via cannula during 2 min. The reaction was stirred for 1 h at −90 °C, then quenched with 1-ethylpiperidine (0.12 mL, 0.84 mmol) by syringe. The reaction mixture was allowed to warm to room temperature and then concentrated. The residue was subjected to column chromatography with EtOAc−hexane (1:15) as eluent to give **10b** (13 mg, 15%) as colorless oil and then **10a** (32 mg, 36%) subsequently as a sticky white solid. Compound **10a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (dt, J_d = 8.4 Hz, J_t = 2.0 Hz, 2H), 7.39 (dt, J_d = 8.4 Hz, J_t = 2.0 Hz, 2H), 6.70 (dd, J = 17.6, 10.8 Hz, 1H), 5.76 (dd, J = 17.6, 0.8 Hz, 1H), 5.26 (dd, J = 10.8, 0.8 Hz, 1H), 5.18 (s, 1H), 3.61 (s, 3H), 2.83 (ddd, J = 14.8, 13.6, 4.0 Hz, 1H), 2.42 (m, 1H), 2.27 (ddd, J = 14.8, 4.8, 3.2 Hz, 1H), 1.89 (m, 1H), 1.70−1.83 (m, 4H), 1.63 (m, 1H), 1.15−1.32 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.9, 137.9, 136.3, 126.0, 125.6, 114.5, 103.9, 96.1, 83.6, 55.9, 45.4, 37.5, 33.4, 32.5, 27.1, 25.2, 23.1. Compound **10b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dt, J_d = 8.4 Hz, J_t = 2.0 Hz, 2H), 7.40 (dt, J_d = 8.4 Hz, J_t = 2.0 Hz, 2H), 6.71 (dd, J = 17.6, 10.8 Hz, 1H), 5.76 (dd, J = 17.6, 0.8 Hz, 1H), 5.26 (dd, J = 10.8, 0.8 Hz, 1H), 5.14 (d, J = 1.2 Hz, 1H), 3.65 (s, 3H), 2.78 (ddd, J = 14.4, 13.2, 3.6 Hz, 1H), 2.30 (ddd, J = 14.4, 4.4, 3.2 Hz, 1H), 1.88−1.99 (m, 2H), 1.60−1.82 (m, 6H), 1.17−1.34 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.2, 137.9, 136.3, 126.0, 125.5, 114.5, 105.1, 105.0, 83.8, 57.2, 47.4, 39.1, 35.6, 30.8, 26.8, 25.0, 23.8. Due to the tendency of styryl systems to polymerize, styryl trioxane **10** was used immediately in the next step.

Preparation of *p*-Styryl Trioxane 10 More Conveniently at −78 °C. A 100 mL three-necked round-bottom flask equipped with dispersion bubbler gas inlet, gas outlet, and a septum was charged with *p*-styryl ketone **9** (*E*/*Z* = ∼1/1, 101 mg, 0.36 mmol) in dry CH_2Cl_2 (35 mL), and methylene blue (1.3 mg) was added. To the solution maintained at −78 °C by a dry ice in acetone bath was bubbled dry air (flow rate = ∼240 mL/min) with a 250W IR lamp (General Electric) at 1 in. distance from the reaction flask. TLC analysis after 20 min showed no starting material, at which time the IR lamp was removed. The air bubbler and outlet also were removed, and the reaction was placed under Ar atmosphere. A precooled (−78 °C) solution of Me_3SiOTf (TMSOTf, 0.09 mL, 0.47 mmol) in CH_2Cl_2 (5 mL) was added slowly via cannula during 2 min. The reaction was stirred for 1 h at −78 °C, then quenched with 1-ethylpiperidine (0.15 mL, 1.1 mmol) by syringe. The reaction mixture was allowed to warm to room temperature and then concentrated. The residue was subjected to column chromatography with EtOAc−hexane (1:15) as eluent to give **10b** (13 mg, 11%) as colorless oil and then **10a** (38 mg, 33%) subsequently as a sticky white solid. Due to the tendency of styryl systems to polymerize, styryl trioxane **10** was used immediately in the next step.

Preparation of *p*-Carboxyphenyl Trioxane 8a. A solution of *p*-styryl trioxane **10a** (215 mg, 0.68 mmol) and $KMnO_4$ (430 mg) in acetone (40 mL) was stirred for 1 h (no starting material by TLC) at room temperature, generating a precipitate. The precipitate was filtered and washed with more acetone, then it was dissolved in MeOH (50 mL). The filtrate was rotary evaporated, and the residue was dissolved in water (7 mL). The aqueous solution was acidified with aqueous 0.5 N HCl solution to pH 2, generating a white solid, which was filtered and recrystallized from MeOH/H_2O at room temperature to give 122 mg (56%) of **8a**. Mp 164.5−165.5 °C (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 5.26 (s, 1H), 3.56 (s, 3H), 2.80 (ddd, J = 14.4, 12.8, 3.6 Hz, 1H), 2.30 (m, 1H), 2.18 (ddd, J = 14.4, 4.4, 2.4 Hz, 1H), 1.86 (m, 1H), 1.58−1.76 (m, 5H), 1.24−1.37 (m, 3H), 1.11 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 144.4, 138.8, 130.5, 126.0, 105.4, 97.6, 85.1, 56.3, 47.0, 39.1, 34.7, 33.6, 28.4, 26.6, 24.1. IR (neat) 3382, 2927, 1595, 1556, 1409, 1100, 1043, 1012, 784 cm^{-1}. Anal. calcd for $C_{18}H_{22}O_6$: C 64.66, H 6.63, found: C 64.61, H 6.57.

Preparation of *p*-Carboxyphenyl Trioxane 8b. A solution of *p*-styryl trioxane **10b** (20 mg, 0.063 mmol) and $KMnO_4$ (40 mg) in acetone (4 mL) was stirred for 0.5 h (no starting material by TLC) at room temperature, generating a precipitate. The precipitate was filtered and washed with more acetone, then it was dissolved in MeOH (4 mL). The filtrate was rotary evaporated, and the residue was dissolved in water (2 mL). The aqueous solution was acidified with aqueous 0.5 N HCl solution to pH 2, which was concentrated. The residue was recrystallized from MeOH/EtOAc at room temperature to give 9 mg (45%) of **8b**. Mp 151−153 °C (white solid). ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 5.13 (s, 1H), 3.65 (s, 3H), 2.78 (dt, J_d = 3.6 Hz, J_t = 14.0 Hz, 1H), 2.24 (dt, J_d = 14.4 Hz, J_t = 3.8 Hz, 1H), 1.85−2.00 (m, 2H), 1.55−1.79 (m, 7H), 1.14−1.28 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 175.8, 139.7, 132.1, 130.5, 126.0, 106.6, 106.4, 84.9, 57.6, 40.4, 36.8, 32.1, 29.9, 28.1, 26.4, 25.1. IR (neat) 3401, 2931, 2858, 1598, 1559, 1413, 1276, 1206, 1137, 1104, 1014, 786 cm^{-1}. Anal. calcd for $C_{18}H_{22}O_6$: C 64.66, H 6.63, found: C 64.71, H 6.55.

Toxicity Testing. Compound **7a** and artelinic acid were dissolved in phosphate-buffered saline (Life Technologies); compound **8a** and arteether were formulated in a vegetable oil/aqueous emulsion. Groups of five male Sprague−Dawley rats (about 165 g at initiation of study) were dosed intravenously by tail vein once daily for 14 consecutive days with 26 μmol/kg/day artelinic acid (limit of solubility); 160 μmol/kg/day arteether, or 40, 80, or 160 μmol/kg/day **7a** or **8a**. Doses were administered at 10 mL/kg (**7a**, **8a**, arteether, controls) or 20 mL/kg (artelinic acid). Each dose was administered to 5 rats. Animals were evaluated at least once daily during dosing for mortality or signs of morbidity. Body weights were obtained before dosing, on days 1, 4, 8, and 12 of dosing, and on day 15 after the start of dosing. Blood was obtained from the retro-orbital sinus on day 8 and at scheduled necropsy on day 15, and was analyzed for red cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white cell count and differential, platelet count, reticulocyte count, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, urea nitrogen, creatine phosphokinase, creatinine, glucose, total protein, albumin, total bilirubin, calcium, chloride, cholesterol, globulin, phosphorus, potassium, and sodium. On day 15, animals were sacrificed by sodium pentothal overdose and perfused transcardially with 10% formalin. Brain, kidneys, liver, and eyes were harvested, weighed, and fixed for histopathologic examination.

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