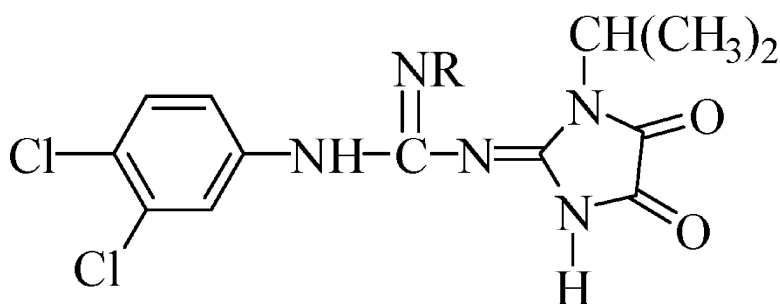


Unambiguous Synthesis and Prophylactic Antimalarial Activities of Imidazolidinedione Derivatives

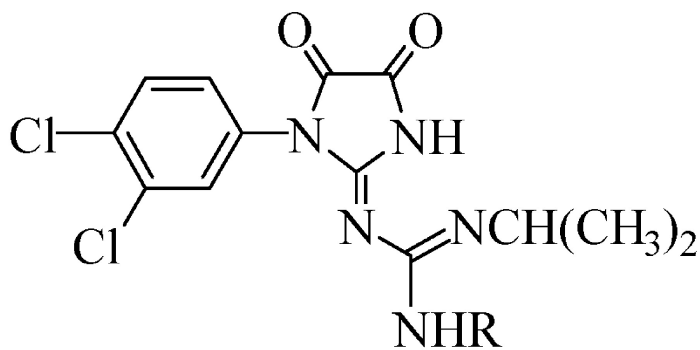
Quan Zhang, Jian Guan, John Sacci, Arba Ager, William Ellis, Wilbur Milhous, Dennis Kyle, and Ai J. Lin

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Unambiguous Synthesis and Prophylactic Antimalarial Activities of Imidazolidinedione Derivatives

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Received May 4, 2005

WR182393, a guanidinoimidazolidinedione derivatives with potent causal prophylactic anti-malarial activity by intramuscular injection, was previously prepared by treatment of chlorproguanil and diethyl oxalate, yielding a mixture of two closely related isomers. Poor solubility of the mixture made the separation and purification impossible. To overcome the separation problem, new and facile unambiguous syntheses of the two active components were reported. The new synthetic methods facilitate the synthesis of not only the active components, but also their derivatives. To search for compounds with good oral efficacy, a series of carbamate derivatives of the active components were prepared by the new procedure, many of which showed profound causal prophylactic antimalarial activity against *Plasmodium yoelii* in mouse by oral administration.

Introduction

The current global situation with respect to malaria indicates that about two billion people are exposed to the disease. Each year between 100 and 200 million new cases of infection are reported, and approximately 1–2 million people die due to malaria.^{1,2} The situation is rapidly worsening, mainly due to the nonavailability of effective drugs and development of drug resistance to the existing first-line drugs, such as chloroquine and pyrimethamine.^{3–5} In addition to the drug resistance of the first-line antimalarial drugs, the usefulness of many newer antimalarial drugs was impaired by their side effects. Multiple drug resistance in *Plasmodium falciparum* malaria continues to pose special problems for targeting the blood stages of malaria. Our product development teams for malaria prophylaxis are placing emphasis on developing new chemical entities with true causal prophylactic or radical curative properties, stopping malaria before blood stages emerge and cause clinical disease. With the exception of quinoline esters,⁶ only the 8-aminoquinoline drugs such as primaquine or tafenoquine⁷ have activity against the liver stages of *Plasmodium vivax* and *P. falciparum* malaras. However, the 8-aminoquinoline drugs caused serious lethal hemolytic side effects in glucose-6-phosphate dehydrogenase (G6PD) deficient patients. Therefore, there is an eminent need for new and safe antimalarial drugs to combat the parasites and protect the tourists traveling in the epidemic areas of the world.

The imidazolidinedione program has its origins in the finding that WR182393, a mixture of compounds **1** and **2** (Figure 1), has radical curative and causal prophylactic activity in rhesus monkeys infected with *Plasmodium cynomolgi*⁸ administered by intramuscular injection.

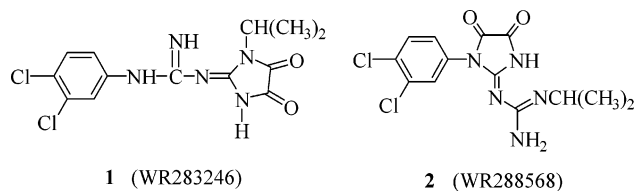


Figure 1.

tion. Although WR182393 was one of the few non-8-aminoquinolines active in the rhesus monkey test model, an active discovery program based on this hit was not pursued. Primarily, this was due to the compound's poor oral bioavailability and the program's emphasis on development of tafenoquine (8-aminoquinoline derivative antimalarial) in the early 1990s. However, the unique activity of WR182393 observed in the rhesus monkey model recently led to its inclusion as a priority within the antimalarial lead optimization program of Walter Reed Army Institute of Research. To continue our earlier efforts on in vivo efficacy experiments, the recent priority was characterization of the chemical properties of WR182393 and the search for derivatives with good oral prophylactic antimalarial activity.

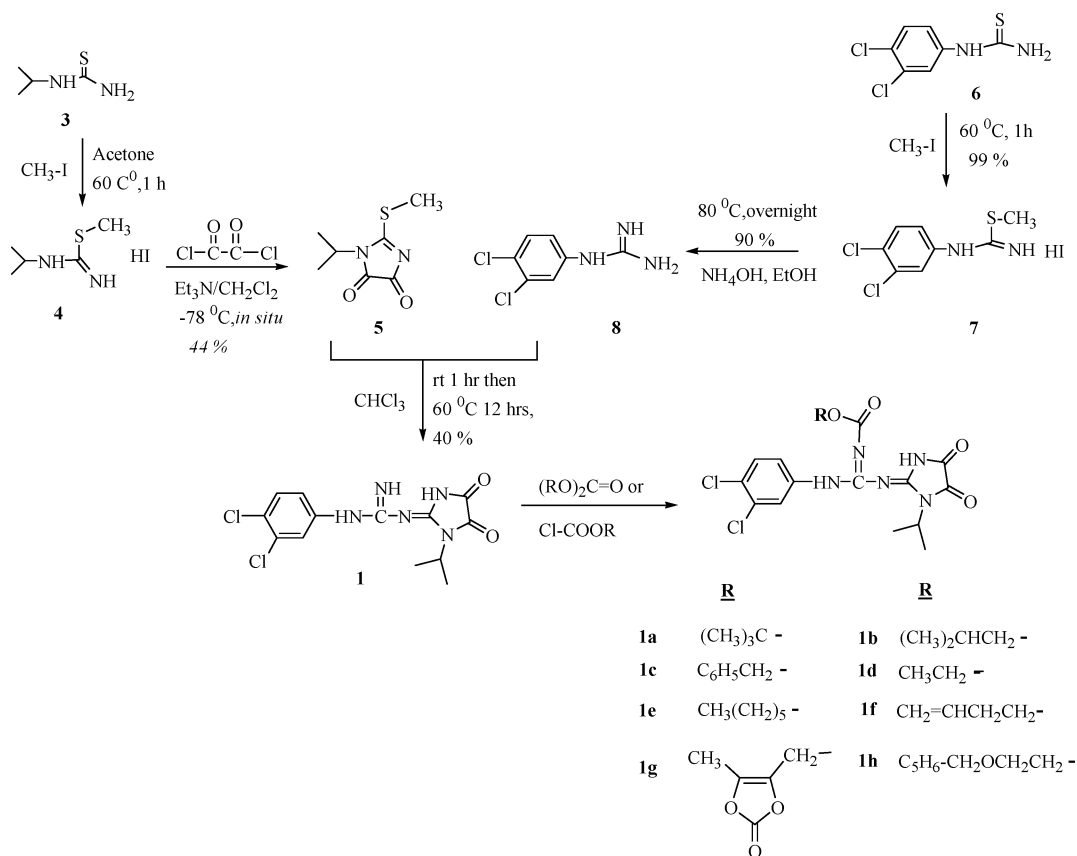
Initially, the test material WR182393 was shown to be a mixture of two products and the starting material, chlorproguanil, with relative proportions of each component in the active mixture estimated to be 54:44:2 of compound **1**, compound **2**, and chlorproguanil, respectively. This was not surprising since WR182393 was prepared by treatment of chlorproguanil with diethyl oxalate, which could result in six different derivatives.⁹ Because of the poor solubility, small-scale purification of the mixture is very difficult, and large-scale purification of the biguanide is almost impossible. Consequently, the structure determination of the components was achieved by carbamate derivatization of the mixture, which enhanced the solubility and facilitated the separation and structural identification of the compo-

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Scheme 1



nents. The structures of the two key active new chemical entities are shown in Figure 1. The results of our efforts to identify components of WR182393 are detailed in a recent publication.⁹ During the process of structure determination of the active components, two carbamate derivatives, **1a** and **2b**, were prepared and showed profound causal prophylactic activity against *P. cynomolgi* in rhesus monkey by intramuscular injection; however, no oral activity was demonstrated in the same test.

In this study, we report the development of two new, facile, and unambiguous methods for the preparation of compounds **1** and **2**, the active components of the mixture. The new procedures provide facile approaches to prepare not only the active components **1** and **2**, but also the new derivatives or analogues of these two lead compounds.

Oral efficacy is an essential consideration in search of prophylactic drugs. The causal prophylactic antimalarial activity of WR182393 in animal models was demonstrated only when the drug was administered by intramuscular injection. No protection of the malaria-infected animals was observed when WR182393 was given orally. To search for compounds with good oral efficacy, a series of carbamate derivatives of the active components were prepared by the new procedure, many of which showed profound causal prophylactic antimalarial activity against *P. yoelii* in mouse by oral administration.

Chemistry

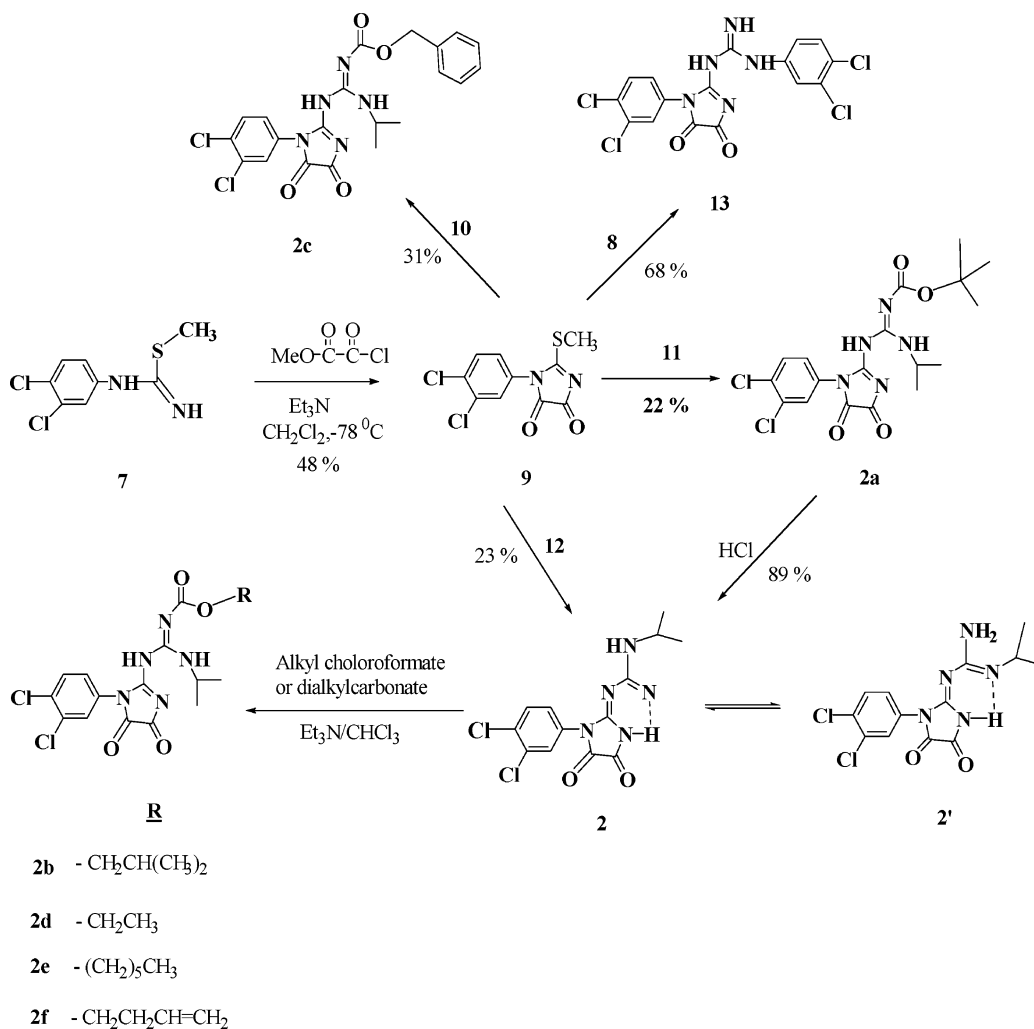
The approach for unambiguous synthesis of **1** involved heating 1-isopropyl-2-methylsulfanyl-1*H*-imidazole-4,5-

dione (**5**) and *N*-(3,4-dichlorophenyl)guanidine (**8**) in chloroform overnight to furnish the yellowish product in 40% yield (Scheme 1).¹⁰ The intermediate **5** was obtained in two steps by methylation of isopropylthiourea **3** with methyl iodide to generate *S*-methylisothiuronium **4**, which was treated with oxalyl chloride under the catalysis of triethylamine to give **5** in 56% yield.^{11,12} Likewise, the synthesis of 3,4-dichlorophenylguanidine (**8**) involved methylation of 3,4-dichlorophenylthiourea **6** with CH₃I to give the corresponding *S*-methylisothiuronium salt **7**, followed by amination to give **8** in 90% yield. Carbamates of compound **1** were prepared by treatment of **1** with either alkyl chloroformate or dialkyl carbonate to give carbamates **1a–h**.

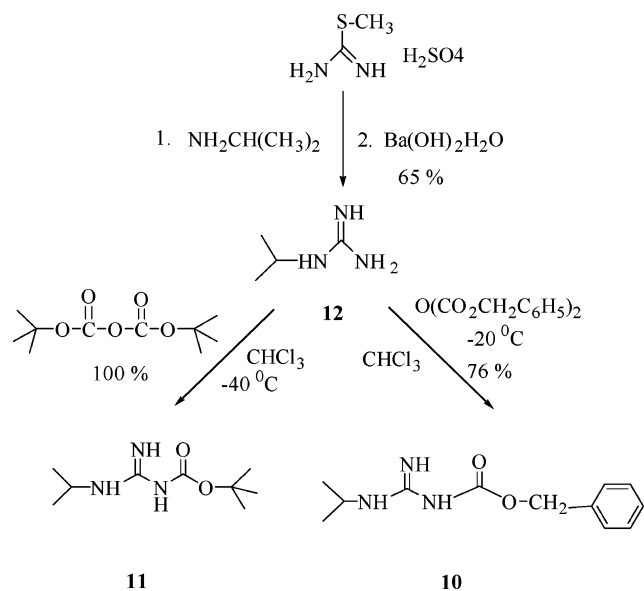
Compound **2** was prepared either by reaction of compound **9** with isopropylguanidine (**12**) or with *t*-Boc isopropylguanidine (**11**) followed by acid hydrolysis of the *t*-Boc carbamate **2a**, as shown in Scheme 2. These two approaches gave a similar total yield, and the product is identical in melting point, IR, and NMR spectrum. Since *t*-Boc guanide **11** is soluble in organic solvent and isopropylguanidine **12** is much more soluble in water than in organic solvent, it is easier to handle the former than the latter during the experiments. Compound **9**, the key intermediate, was produced in good yield by treatment of *S*-methylisothiuronium **7** with methyl chlorooxacetate (methyl oxalyl chloride) in CH₂Cl₂ under Et₃N catalysis at -78°C . The carbamates **2a**, **2c**, and **13** were prepared by the same procedure, treating intermediate **9** with compound **10**, **11**, and **8**, respectively, as shown in Scheme 2.

The key intermediate, *N*-isopropyl-*N'*-(*tert*-butylcarbonyl)guanidine **11**, was obtained in good yield by

Scheme 2



Scheme 3



treatment of isopropylguanidine (**12**) with di-*tert*-butyl carbonate (Scheme 3). Compound **12** was prepared, in turn, by amination of a commercially available product, *S*-methylisothiuronium sulfate, followed by conversion of the isopropylguanidine salt to free base **12** by treatment with $\text{Ba}(\text{OH})_2$.¹³ The other intermediate **10**, *N*-

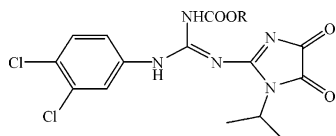
(benzylcarbonyl)-*N'*-isopropylguanidine, was prepared by treatment of **12** with dibenzyl carbonate.

Although the NMR spectra of compound **1** in CDCl_3 showed a pure single compound, the NMR spectra of compound **2** indicated the existence of two tautomers, **2** and **2'**, in ratio of about 3:1, as indicated by the integration of two sets of doublets at 1.14 and 1.09 ppm for the methyl protons of the isopropyl group.¹⁴

Both imidazolidinediones **1** and **2** are sparingly soluble in organic solvents and water, especially compound **2**. Heating is required to dissolve compound **2** in DMSO or DMF.

Antimalarial Activity

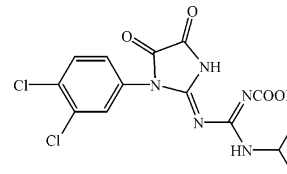
The causal prophylactic activities of the new imidazolidinedione carbamates (**1a-h** and **2a-f**) were assessed in mouse infected with sporozoites of *Plasmodium yoelii*. The results were shown in Tables 1 and 2. The lead molecule **1** and its carbamate derivatives are much less active than lead molecule **2** and its corresponding carbamates. While compound **2** provided 100% protection (5/5) at 20 mg/kg administered orally and protected two out of five mice in the 5 mg/kg group, compound **1** showed no protection activity up to 160 mg/kg. Likewise, the carbamate derivatives of **2** are much more active than those of compound **1**. Among the carbamate derivatives of compound **1**, only *t*-Boc (**1a**), isobutyl (**1b**),

Table 1. Oral Prophylactic Activity in *P. yoelii* Sporozoites Infected Mouse

compd	R ^a	dose (mg/Kg)	no. mice protected/total
1		160	0/5
		40	0/5
		10	0/5
1a	C(CH ₃) ₃	160	5/5
		40	2/5
		10	0/5
1b	CH ₂ CH(CH ₃) ₂	160	5/5
		40	2/5
		10	0/5
1c	CH ₂ C ₆ H ₅	160	0/5
		40	0/5
1d	CH ₂ CH ₃	160	5/5
		40	4/5
		10	2/5
1e	(CH ₂) ₅ CH ₃	160	5/5
		40	2/5
		10	0/5
1f	CH ₂ CH ₂ C=CH ₂	160	1/5
		40	0/5
		10	0/5
1g		160	3/5
		40	2/5
		10	2/5
		2.5	0/5
1h	CH ₂ CH ₂ OCH ₂ C ₆ H ₅	160	1/5
		40	0/5
		10	0/5
		10	0/5
primaquine		160	5/5
		40	2/5
		10	0/5
		10	0/5
arteether		160	0/5
		40	0/5
		10	0/5
		10	0/5
tafenoquine		160	toxicity death
		40	5/5
		10	5/5
		5	5/5
		2.5	3/5

and ethyl (**1d**) carbamates showed 100% protection of the high dose (160 mg/kg) group. Carbamate **1c** showed no protection (0/5) at 160 mg/kg, and carbamates **1e**, **1f**, and **1h** protected only one out of five treated mice (1/5) at the same dose. Whereas the isobutyl carbamate of compound **2** (**2b**) protected 100% of the treated mice at a dosage as low as 5 mg/kg, the minimum dose for 100% protection of isobutyl carbamate **1b** is >40 mg/kg. Likewise, while t-Boc **2a** protected 4/5 mice at dosage of 5 mg/kg, t-Boc **1a** protected only 2/5 mice at a higher dose of 40 mg/kg. However, ethyl carbamates **1d** and **2d** showed comparable causal prophylactic activity at all dose levels tested, showing 2/5 and 1/5 mice protection, respectively, at 10 mg/kg.

The test in this mouse model against *P. yoelii* may yield false-positive results because of the relatively short pre-erythrocytic stage of the parasite (2 days) and the unknown biological half-life of the test compounds. It is, therefore, considered to be a test for presumptive activity, and a positive result must be confirmed in another system, such as *P. cynomolgi* in rhesus mon-

Table 2. Oral Prophylactic Activity in *P. yoelii* Sporozoites Infected Mouse

compd	R	dose (mg/kg)	no. mice protected/total
2		80	5/5
		40	5/5
		20	5/5
		10	3/5
		5	2/5
2a	C(CH ₃) ₃	160	5/5
		40	4/5 ^a
		10	4/5
		5	4/5
		2.5	2/5
2b	CH ₂ CH(CH ₃) ₂	1.25	2/5
		160	5/5
		40	5/5
		10	5/5
		5	5/5
2c	CH ₂ C ₆ H ₅	2.5	1/5
		1.23	0/5
		160	3/5
		40	2/5
		10	2/5
2d	CH ₂ CH ₃	5	3/5
		2.5	0/5
		80	5/5
		40	5/5
		20	3/5
2e	(CH ₂) ₅ CH ₃	10	1/5
		80	5/5
		40	3/5
		20	4/5
		10	2/5
2f	CH ₂ CH ₂ C=CH ₂	5	2/5
		ND ^b	

^a One dead. ^b ND, not dosed.

keys, a gold standard test. Further, if all of the sporozoites or exoerythrocytic (EE) stage parasites are killed, then blood stream parasites will not appear. The number of EE stages that are killed will be reflected in the subsequent reduction in parasitemia. However, the strain of *P. yoelii* (17XNL) used in the model is nonlethal. The mice developed high parasitemia in the first 6 days after inoculation with sporozoites and eventually self-cured, and most survive even without treatment. To conclude that the mice are cured by the drug may or may not be true. Therefore, the mouse model can only be used as a preliminary screen and the results can only be used as one criterion for selection of candidates for further tests in the monkey model. This was exemplified by the fact that, although compound **2b** showed superior efficacy over compound **1a** in mouse test, the latter was more active than the former in tests against *P. cynomolgi*^{15,16} in rhesus monkey, which has been demonstrated to give results parallel to that observed in clinics. Therefore, good activity in the mouse test observed in this study will be further confirmed in the gold standard rhesus monkey model. The drug distribution and metabolism of rhesus monkey is generally much closer to that of human than other commonly used animals.

Table 3. In Vitro Antimalarial Activity against *P. falciparum*

drug	parasites	IC ₅₀ (ng/mL)	IC ₉₀ (ng/mL)
mefloquine	W2	1.86	4.01
	D6	4.90	13.44
	TM91C235	21.63	44.01
chloroquine	W2	125.71	198.69
	D6	4.1754	5.33
	TM91C235	34.57	55.24
WR182393	W2	2664.69	10894.01
	D6	2971.72	12066.59
	TM91C235	2589.26	11351.15
1	W2	2380.95	11918.96
	D6	1627.04	9365.79
	TM91C235	2541.89	11874.15
2	W2	6663.44	12282.73
	D6	7182.39	9631.66
	TM91C235	5384.93	9981.42

It is interesting to note that while carbamates **1a** and **2b** possess high oral prophylactic activity against *P. yoelii* in mouse model, no oral causal prophylactic activity was observed in rhesus monkey model against liver stage *P. cynomolgi* at a dose up to 60 mg/kg.⁹ The cause of differences in oral efficacy observed in rhesus monkey and mouse may relate to the difference in their stomach acidity. The facts that the pH of monkey stomach (1–3) is lower than that of mouse (pH 3.1–4.5) and that the half-life of carbamates **1a** and **2b** in simulated stomach acid (0.01 N HCl in ethanol) is only about 30 min may account for the species differences in oral activity observed.^{17,18} The major hydrolysis product of **1a** and **2b** in simulated stomach acid was identified as the lead compounds **1** and **2**, respectively, both of which are sparingly soluble in organic solvents and water. The difference in stomach acidity of mouse and monkey leads to the difference in rate of hydrolysis of carbamates. The higher pH of mouse stomach acid may account for the lower rate of carbamate hydrolysis and thus better bioavailability and higher oral efficacy.

It is further noted that neither the lead compounds **1** and **2** nor their carbamates showed activity against the blood stage parasites in the in vitro assay against *P. falciparum* (Table 3). Likewise, compounds **1a**, **1b**, **1c**, **1d**, **1e**, and **1f** were inactive in the Thompson test against blood stage parasite *Plasmodium berghei* from 20 to 320 mg/kg by sc or po, although moderate activity in EE tests against *P. yoelii* (liver stage model) were observed (Table 1). To the best of our knowledge, this is the first class of chemoprophylaxis antimalarial agent that is totally devoid of activity against blood stage malaria. Although it is a general belief that an ideal therapeutic drug should be effective for both prophylaxis and treatment, drugs with only prophylactic activity, such as the compounds under this study, will have much less exposure to the parasites in the field than the treatment drugs. Thus, development of drug resistant parasites to drugs with prophylaxis only is less likely to be a problem.

In summary, two facile and unambiguous synthetic procedures were developed to prepare both active components of WR182393. The new methods were also utilized to make a series of carbamate derivatives, some of which showed good oral efficacy in mouse infected with sporozoites of *P. yoelii*. The causal prophylactic activity of the best compound demonstrated in the

mouse model will be confirmed in rhesus monkey against *P. cynomolgi*.

Experimental Section

Chemistry. Melting points were determined on a Mettler FP62 melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed using EM Reagents 0.25 mm HPTLC-HLF normal-phase 150 μ m silica gel 60-F plates (Analtech, Newark, DE). Liquid chromatography was performed using a force flow (flash chromatography) Horizon HPFC system (Biotage, Charlottesville, VA) with Flash 25M or 40M cartridges (KP–Siltm Silica, 32–63 μ m, 60 Å). Preparative TLC was performed using silica gel GF Tapered Uniplates (Analtech, Newark, DE). Infrared spectra were recorded on a Bio-Rad FTS 3000 spectrophotometer (Bio-Rad Laboratories, Cambridge, MA) and are reported in reciprocal centimeters (cm⁻¹). ¹H NMR and ¹³C NMR spectra were recorded in deuteriochloroform, unless otherwise noted, on a Bruker AC300 or Bruker Avance 600 spectrometer (Bruker Instruments, Inc., Wilmington, DE). Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Where analyses are indicated by symbols of the elements, the analytical results obtained were within $\pm 0.4\%$ of the theoretical values.

1-Isopropyl-2-methylsulfanyl-1H-imidazole-4,5-dione (5). To isopropyl thiourea **3** (2.364 g, 20.0 mmol) in 40 mL of dry acetone was added iodomethane (1.87 mL, 30.0 mmol) dropwise at room temperature. After the addition was completed, the mixture was refluxed for 1 h, and the solvent was evaporated to dryness under the reduced pressure to give a gum. The gummy HI salt was suspended in 200 mL of dry CH₂Cl₂ and to the suspension was added triethylamine (11 mL, 80.0 mmol). The mixture was stirred for 30 min at room temperature and cooled to -78 °C with a dry ice/acetone bath. To the mixture was added dropwise oxalyl chloride (12 mL of 2 M solution, 24 mmol), and the mixture stirred at -78 °C for 1 h. The reaction was quenched by addition of water to decompose the excess oxalyl chloride; the solvent was dried over Na₂SO₄ and evaporated to dryness in vacuo. Recrystallization of the solid product with CHCl₃/hexanes mixed solvent yielded intermediate **5** as a yellow needle (1.62 g, 44%), mp 193.5 °C. ¹H NMR (600 MHz, CDCl₃): δ 4.19 (m, 1H), 2.76 (s, 3H), 1.48 (d, J = 6.6 Hz, 6H). ¹³C NMR: δ 191.64, 167.18, 158.49, 48.62, 20.06, 14.64; IR: 1759, 1740, 1450, 1303, 1050 cm⁻¹. MS (m/z): 172, 144, 83. Anal. (C₇H₁₀N₂O₂S): C, H, N, S.

1-(3,4-Dichlorophenyl)-2-methylisothiurea HI Salt (7). To a solution of 3,4-dichlorophenylthiourea (**6**) (5.00 g, 22.61 mmol) in 80 mL of anhydrous acetone was added iodomethane (2.11 mL, 33.92 mmol), and the resultant solution was heated at reflux for 1 h. The solvent was removed under reduced pressure, and the residue was suspended in EtOAc. The white solid product was collected, washed with fresh solvent, and dried in vacuo to give HI salt (**7**) (8.119 g, 22.37 mmol, 99%) as a white solid, mp 160 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 7.80 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.4 Hz, 1H), 7.37 (dd, J = 8.6 and J = 2.4 Hz, 1H), 2.78 (s, 3H). ¹³C NMR: δ 170.64, 136.24, 132.94, 132.63, 131.68, 128.95, 123.19. IR: 1628, 1576, 1541, 1464, 1306, and 1124 cm⁻¹. Anal. (C₈H₉Cl₂IN₂S): C, H, Cl, N, S.

To the water solution of 1-(3,4-dichlorophenyl)-2-methylisothiurea hydroiodide (**7**) was added saturated Na₂CO₃ solution at ice-cold temperature till the pH reached about 9–10. The reaction mixture was extracted with CHCl₃ three times. The chloroform extracts were combined and dried over Na₂SO₄. The solvent was removed in vacuo to give crude free base of **7** as a white solid in quantitative yield, mp 166 °C. The product was pure enough for further reaction. ¹H NMR (600 MHz, CDCl₃) δ 7.34 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 2.4 Hz, 1H), 6.75 (dd, 8.4 and 2.4 Hz, 1H), 4.58 (s, 2H), 2.42 (s, 3H). ¹³C NMR: δ 157.14, 148.74, 133.24, 131.31, 126.80, 124.23, 122.09, and 14.16.

***N*-(3,4-Dichlorophenyl)guanidine (8).** To the solution of **7** (2.29 g, 6.31 mmol) in 23 mL of ethanol was added 6.87 mL of NH_4OH . The reaction mixture was stirred at room temperature for 10 min, and the clear solution was refluxed for 12 h. The solvent was removed under reduced pressure and the residue was suspended in saturated Na_2CO_3 solution. The solid was collected, washed sequentially with H_2O and Et_2O and dried to give the title compound as a white solid (1.16 g, 90%), mp 208 °C. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 7.39 (d, $J = 8.6$ Hz, 1H), 7.02 (d, $J = 2.4$ Hz, 1H), 6.79 (dd, $J = 8.6$ and 2.4 Hz, 1H), 5.79 (br s, 3H). ^{13}C NMR: δ 155.38, 130.69, 130.28, 124.30, and 123.50. IR: 1684, 1624, 1559, 1472, 1380, 1328, 869, 818, 671 cm^{-1} . MS (m/z): 204 (M^+), 187, 162, 127, and 90. The compound was pure enough to be used for further reactions without purification.

1-(3,4-Dichlorophenyl)-2-methylsulfanyl-1*H*-imidazole-4,5-dione (9). The reaction solution of 1-(3,4-dichlorophenyl)-2-methylisothiourea (**7**) (6.6 g, 28.3 mmol) and triethylamine (7.5 mL, 56.5 mmol) in 200 mL of dry CH_2Cl_2 was cooled to -78 °C with an acetone/dry ice bath. To the solution was added dropwise methyl oxalyl chloride (5.2 mL, 56.5 mmol). The resulting brown reaction mixture was stirred at -78 °C for 2 h and quenched with water. The mixture was extracted with CHCl_3 three times and the CHCl_3 extracts were combined, dried over Na_2SO_4 , and evaporated to dryness under the reduced pressure. The crude product was purified by recrystallization with hexanes/ CHCl_3 mixed solvent to yield **9** as yellow crystals (4.0 g, 16.12 mmol, 48%). ^1H NMR (300 MHz, CDCl_3): δ 7.63 (d, $J = 8.7$ Hz, 1H), 7.46 (d, $J = 2.4$ Hz, 1H), 7.20 (dd, $J = 8.7$ and 2.4 Hz, 1H), 2.76 (s, 3H). ^{13}C NMR: δ 179.00, 158.00, 135.14, 134.19, 129.97, 131.67, 129.07, 126.36, 14.44. MS (m/z): 289 (M^+), 286, 187, 159, and 124. Anal. ($\text{C}_{10}\text{H}_6\text{Cl}_2\text{O}_2\text{S}$) C, H, Cl, N.

***N*-(Benzylcarbonyl)-*N'*-isopropylguanidine (10).** To the solution of isopropylguanidine (**12**) (0.50 g, 4.95 mmol) in DMF (20 mL) was added with stirring at -20 °C a solution of dibenzyl dicarbonate (0.644 g, 2.25 mmol) in 20 mL of CHCl_3 . The mixture was stirred for an additional 2 h after the addition was completed and allowed to stir at room temperature overnight. After being quenched with water, the mixture was extracted with CHCl_3 , washed with water (4 \times 100 mL), and dried over Na_2SO_4 . The solvent was removed under reduced pressure to afford the product **10** as a colorless crystal (0.400 g, 1.702 mmol, 76%). ^1H NMR (300 MHz, CDCl_3) δ 7.40–7.25 (m, 5H), 5.08 (s, 2H), 3.57 (m, 1H), 1.22 (d, $J = 6.0$ Hz, 6H). ^{13}C NMR: δ 163.80, 160.47, 137.33, 128.17, 127.62, 127.45, 66.05, 43.20, and 22.63.

***N*-Isopropyl-*N'*-(*tert*-butylcarbonyl)guanidine (11).** To the solution of isopropylguanidine (**12**) (1.33 g, 13.17 mmol) in DMF (30 mL) was added, in small portions, at room temperature a solution of di-*tert*-butyl dicarbonate (1.435 g, 6.584 mmol) in 40 mL of CHCl_3 , and the reaction mixture was stirred at room temperature overnight. After quenching with water (20 mL), the mixture was extracted with CHCl_3 three times. The chloroform extracts were combined, washed with water (4 \times 50 mL), and dried over Na_2SO_4 . The solvent was evaporated to dryness under reduced pressure to afford the pure crude product **11** as a colorless liquid (1.32 g, 6.58 mmol, 100%). The crude product was used for further reactions without purification. ^1H NMR (300 MHz, CDCl_3): δ 3.51 (m, $J = 6.3$ Hz, 1H), 1.43 (s, 9H), 1.20 (d, 5.3 Hz, 6H). ^{13}C NMR: δ 63.81, 160.49, 77.84, 43.16, 29.70, and 22.88.

Isopropylguanidine (12). A solution of *S*-methylisothiourea sulfate (65.9 g, 250 mmol) in water (100 mL) was cooled with an ice/salt bath. To the solution was added isopropylamine (42.5 mL, 500 mmol) dropwise with stirring. The reaction mixture was allowed to stir at room temperature for 16 h after the addition of amine was completed and was then refluxed for 4–5 h. The solution was evaporated to dryness under the reduced pressure and the residue was crystallized from 95% EtOH , to give isopropylguanidine sulfate as colorless needles (16.12 g, 81.01 mmol, 32%). ^1H NMR (300 MHz, D_2O): δ 3.72 (m, 1H), 1.27 (d, $J = 6.4$ Hz, 6H). ^{13}C NMR: δ 158.45, 46.50, 24.11; IR: 1675, 1623, 1071 cm^{-1} .

The isopropylguanidine sulfate was converted to its free base by addition of $\text{Ba}(\text{OH})_2 \cdot \text{H}_2\text{O}$, and the barium sulfate formed was removed by centrifugation to give free base **12** as a colorless viscous gel in 65% yield. ^1H NMR (300 MHz, D_2O): δ 3.64 (m, 1H), 1.17 (d, $J = 6.5$ Hz, 6H). ^{13}C NMR: δ 46.34, 24.10.

***N*-(3,4-Dichlorophenyl)-*N'*-[1-(3,4-dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]guanidine (13).** To the solution of the crude dione **9** (0.551 g, 1.907 mmol) in 25 mL of dry CHCl_3 was added *N*-(3,4-dichlorophenyl)guanidine (**8**) (0.389 g, 1.907 mmol) at room temperature. The reaction mixture was heated at 50 °C overnight. The solid was collected and washed sequentially with CHCl_3 and H_2O to afford the compound **13** as a light yellow solid (0.573 g, 1.288 mmol, 68%), mp 220 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.75 (d, $J = 8.3$ Hz, 1H), 7.69 (s, 1H), 7.55–7.40 (m, 3H), 7.15 (d, 7.9 Hz, 1H). ^{13}C NMR: δ 170.54, 169.33, 160.26, 156.74, 136.75, 132.72, 131.15, 131.00, 130.73, 130.52, 129.64, 128.04. MS (m/z) 444 (MH^+), 256, 227, 185. Anal. ($\text{C}_{16}\text{H}_9\text{Cl}_4\text{N}_5\text{O}_2$) C, H, Cl, N.

***N*-(3,4-Dichlorophenyl)-*N'*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (1).** To the solution of dione **5** (0.712 g, 3.83 mmol) in 80 mL of dry CHCl_3 was added guanidine **8** (0.74 g, 3.64 mmol), and the reaction mixture was heated in an oil bath at 50 °C for 16 h. The solid precipitates were collected and washed sequentially with H_2O , CH_3OH , and CHCl_3 to afford the compound **1** as a light yellow solid (0.497 g, 40%), mp 233 °C. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 7.77 (d, $J = 2.2$ Hz, 1H), 7.68 (d, $J = 8.6$ Hz, 1H), 7.31 (dd, $J = 8.6$ and 2.2 Hz, 1H), 4.38 (m, 1H), 1.33 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR: δ 172.24, 170.65, 162.14, 160.08, 132.13, 131.76, 126.96, 125.00, 44.44, 20.54. IR: 3277, 3140, 1752, 1725, 1553, 1464, 1317, 996, 808, 754 cm^{-1} . Anal. ($\text{C}_{13}\text{H}_{13}\text{Cl}_2\text{N}_5\text{O}_2$): C, H, N.

***N*-(3,4-Dichlorophenyl)-*N'*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)-*N''*-(*tert*-butylcarbonyl)guanidine (1a).** To the mixture of *N*-(3,4-dichlorophenyl)-*N'*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (**1**) (50 mg, 0.146 mmol) and DMF (5 mL) was added DMAP (1.8 mg), followed by di-*tert*-butyl dicarbonate (127 mg, 0.58 mmol). The reaction mixture was further stirred at room temperature overnight. After being quenched with water, the mixture was extracted with CHCl_3 , dried over Na_2SO_4 , and concentrated. The crude product was purified by recrystallization from $\text{EtOAc}/\text{CHCl}_3$ to furnish **1a** as a yellow needle (15.5 g, 24%), mp 226 °C. ^1H NMR (600 MHz, CDCl_3): δ 7.81 (d, $J = 2.3$ Hz, 1H), 7.50 (d, $J = 8.6$ Hz, 1H), 7.24–7.7.22 (dd, $J = 8.6$ and 2.3 Hz, 1H), 4.42 (m, 1H), 1.57 (s, 9H), 1.37 (d, $J = 4.6$ Hz, 6H). ^{13}C NMR: δ 156.32, 134.82, 133.51, 131.45, 131.03, 126.77, 126.68, 123.55, 86.72, 45.69, 28.42, 20.35. MS (m/z): 442 (M^+), 390, 387, 331, 288, 248, 204. The compound was identical to that prepared from the mixture WR182393 in melting point and NMR.^{9,10}

***N*-(3,4-Dichlorophenyl)-*N'*-(isobutylcarbonyl)-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (1b).** Compound **1b** was prepared by the same method as for preparation of **1a**, using pure compound **1** (1.400 g, 4.094 mmol), to yield a pale yellow solid (1.162 g, 2.629 mmol, 64%). ^1H NMR (300 MHz, CDCl_3) δ 13.10 (s, 1H), 11.13 (s, 1H), 7.81 (d, $J = 2.5$ Hz, 1H), 7.50 (d, $J = 8.6$ Hz, 1H), 7.21 (dd, $J = 8.6$ and 2.5 Hz, 1H), 4.07 (d, $J = 6.9$ Hz, 1H), 2.09–2.03 (m, 1H), 1.37 (d, $J = 6.9$ Hz, 6H), 1.00 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR: δ 173.40, 168.24, 160.28, 155.91, 154.85, 134.28, 133.06, 131.12, 130.67, 126.45, 123.23, 73.97, 45.34, 27.64, 19.95, 18.91. MS (m/z): 442 (M^+), 386, 352, 341, 314, 248, 179, 156, 114. Anal. ($\text{C}_{18}\text{H}_{21}\text{Cl}_2\text{N}_5\text{O}_4$): C, H, N.

***N*-(3,4-Dichlorophenyl)-*N'*-(benzylcarbonyl)-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (1c).** Compound **1c** was prepared by the same method as the preparation of **1a**, using mixture WR182393 as starting material to yield compound **1c** as a pale yellow solid (20%),⁹ mp 235 °C. ^1H NMR (CDCl_3 , 600 Hz) δ 7.81 (1H, s), 7.53 (1H, d, $J = 8.6$), 7.42–7.45 (5H, m), 7.23 (1H, d, $J = 8.6$), 5.32 (2H, s), 4.43 (1H, m), 1.39 (6H, d).

***N*-(3,4-Dichlorophenyl)-*N'*-ethylcarbonyl-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (1d).** To

the mixture of *N*-(3,4-dichlorophenyl)-*N'*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (**1**) (0.050 g, 0.146 mmol) in CHCl₃ (5 mL) was added, at room temperature, first Et₃N (63 μL, 0.045 mmol) and then ethyl chloroformate (42 μL, 0.44 mmol). The reaction mixture was further stirred at room temperature overnight. After being quenched with water, the mixture was extracted with CHCl₃, dried over Na₂SO₄, and evaporated to dryness. The residue was purified by flash silica gel column chromatography using 10% ethyl acetate in hexanes as eluent to furnish **1d** as a white solid (0.0121 g, 20%),⁹ mp 166.4 °C: ¹H NMR (300 MHz, CDCl₃): δ 7.80 (d, *J* = 2.5 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.23–7.7.19 (dd, *J* = 8.6 and 2.5 Hz, 1H), 4.47–4.28(m, 3 H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.36 (d, *J* = 7.0 Hz, 6H). ¹³C NMR: δ 173.40, 168.26, 160.28, 155.89, 154.65, 134.25, 133.06, 131.12, 130.67, 126.44, 123.22, 64.16, 45.35, 19.95, and 14.14. The compound was identical to that prepared from the mixture WR182393 in melting point and NMR.^{9,10}

***N*-(3,4-Dichlorophenyl)-*N'*-(3-hexylcarbonyl)-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (**1e**)**. Compound **1e** was prepared by the same method as for **1a** to give yellow crystals (0.6 g, 31%). ¹H NMR (300 MHz, CDCl₃): δ 7.84 (d, *J* = 2.4 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.23 (dd, *J* = 8.6 and 2.4 Hz, 1H), 4.49–4.40 (m, 1H), 4.32 (t, *J* = 6.7 Hz, 2H), 1.82–1.72 (m, 3H), 1.58 (s, 5H), 1.40 (d, *J* = 6.9 Hz, 6H), 0.93 (t, *J* = 6.4 Hz, 3H). ¹³C NMR δ 173.10, 168.10, 160.50, 155.89, 154.82, 134.27, 133.07, 131.30, 130.67, 126.44, 123.20, 68.29, 45.35, 31.31, 28.38, 25.31, 22.48, 19.95, 13.99. MS (*m/z*): 472, 470 (M⁺), 416, 368, 316, 260, 186, and 156. Anal. (C₂₀H₂₅Cl₂N₅O₄): C, H, N.

***N*-(3,4-Dichlorophenyl)-*N'*-(3-butenylcarbonyl)-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (**1f**)**. Compound **1f** was also prepared by the same method as the preparation of **1a**, using pure compound **1** (100 mg) as starting material to yield compound **1f** as a pale yellow solid (66 mg, 52%). ¹H NMR (300 MHz, CDCl₃): δ 7.83 (d, *J* = 2.5 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.23 (dd, *J* = 8.6 and 2.5 Hz, 1H), 5.89–5.78 (m, 1H), 5.24–5.16 (m, 2H), 4.49–4.42 (m, 1H), 4.40 (t, *J* = 7.1 Hz, 2H), 2.54 (q, *J* = 6.8 Hz, 2H), 1.40 (d, *J* = 6.9 Hz, 6H). ¹³C NMR δ 173.10, 168.10, 160.18, 155.95, 154.81, 134.27, 133.07, 132.57, 131.14, 130.68, 126.45, 123.22, 118.44, 66.90, 45.37, 32.78, 19.95. MS (*m/z*): 442, 440 (M⁺), 351, 267, 191, 155. Anal. (C₁₈H₁₉Cl₂N₅O₄): C, H, N.

Preparation of *N*-(3,4-Dichlorophenyl)-*N'*-(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (1g**)**. A dimethylformamide suspension of compound **1** (200 mg, 0.58 mmol) was treated with (5-methyl-1,3-dioxolene-2-oxo-4-yl)methyl *p*-nitrophenyl carbonate^{19–22} (204 mg, 0.69 mmol) and DMAP (14 mg, 0.11 mmol). The reaction mixture was stirred at room temperature overnight and partitioned in CHCl₃/H₂O. The water layer was extracted several times with chloroform. The chloroform extracts were combined, washed successively with brine and water, dried over Na₂SO₄, filtered, and concentrated to yield crude product as yellow gum (258 mg). The crude product solidified upon treatment in ethyl acetate/hexane (1:1) to give yellow solid **1g** (91 mg, 31%), mp 144 °C. ¹H NMR (CDCl₃, 300 Hz) δ 7.73 (d, *J* = 2.4 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.20 (dd, *J* = 8.6, *J* = 2.4 Hz, 1H), 5.01 (s, 2H), 4.41 (m, 1H), 2.23 (s, 3H), 1.36 (d, *J* = 6.9 Hz, 6H). MS (*m/z*): 497 (M⁺). Anal. (C₁₉H₁₇N₅O₇Cl₂) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N'*-(benzyloxyethylcarbonyl)-*N''*-(1-isopropyl-4,5-dioximidazolidin-2-ylidene)guanidine (**1h**)**. Compound **1h** was prepared by the same method of **1a** to yield compound **1h** as a white crystal (0.4 g, 22%). ¹H NMR (300 MHz, CDCl₃) δ 13.17 (s, 1H), 11.08 (s, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.39–7.29 (m, 5H), 7.21 (dd, *J* = 8.6 and 2.4 Hz, 1H), 4.61 (s, 2H), 4.49–4.42 (m, 3H), 3.78 (t, *J* = 4.7 Hz, 2H), 1.39 (d, *J* = 6.9 Hz, 6H). ¹³C NMR δ 172.50, 167.60, 166.10, 156.04, 155.30, 155.00, 138.00, 137.47, 134.31, 133.04, 131.21, 130.66, 128.54, 128.48, 127.94, 127.85, 127.80, 126.47, 123.25, 73.30, 71.35, 67.16, 66.66, 64.56, 61.92, 45.40, 19.94. MS (*m/z*): 522, 520 (M⁺), 492, 432, 370, 368, 298, 232, and 181.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl]-*N'*-isopropylguanidine (**2**)**. 1-(3,4-Dichlorophenyl)-2-methylsulfanyl-1*H*-imidazole-4,5-dione (**9**) (1.89 g, 6.54 mmol) was dissolved in 10 mL of dried DMF and cooled with an ice bath to 0 °C. To the solution was added dropwise 40 mL of DMF solution containing guanidine **12** (1.369 g, 13.554 mmol). The solution was stirred at room temperature for 2 h after addition and then further stirred at 50 °C for 24 h. The solvent DMF was dried under vacuum and the crude product was suspended in 30 mL of chloroform. The yellow solid was collected and washed sequentially with 3 × 20 mL of CHCl₃ and 10 mL of methanol to afford compound **2** as a white powder (1.588 g, 6.54 mmol, 71%), mp 245 °C. NMR indicates that the compound exists in two tautomeric forms in the CDCl₃ solution. Major tautomer ¹H NMR (600 MHz, DMSO-*d*₆): δ 8.74–8.69 (m, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.76 (d, *J* = 2.2 Hz, 1H), 7.44 (dd, *J* = 8.6 and 2.2 Hz, 1H), 3.73 (m, 1H), 1.14 (d, *J* = 6.2 Hz, 6H). ¹³C NMR: δ 168.60, 162.45, 161.15, 161.09, 134.01, 131.84, 131.48, 131.20, 130.29, 128.86. IR: 1757, 1722, 1645, 1595, 1551, 1535, 1480, 1304, 997, 754 cm⁻¹; MS (*m/z*): 342 (M⁺), 271, 229, 187, 127. Anal. (C₁₃H₁₃Cl₂N₅O₂·0.25H₂O) C, H, Cl, N.

Minor tautomer ¹H NMR (600 MHz, DMSO-*d*₆): δ 8.35 (s, 1H), 7.99 (s, 1H), 7.88 (d, *J* = 7.3 Hz, 1H), 7.42 (dd, *J* = 8.6 and 2.2 Hz, 1H), 1.09 (d, *J* = 6.4 Hz, 6H).

The signals of one of the aromatic protons and the methylene proton of the isopropyl function cannot be identified. The former was overlapping with the signals of the major tautomer and the latter was too weak and too broad to be identified.

Compound **2** was also prepared by an alternative method: A solution of **2a** (0.424 g, 0.959 mmol) in dry CHCl₃ (60 mL) was cooled with an ice bath. To the solution was added dropwise concentrated HCl (2 mL), and the mixture stirred at 0 °C for 1 h and then at room temperature overnight. Saturated Na₂CO₃ was added to basicify the mixture. The white solid was collected, washed successively with CHCl₃ and water, and dried under reduced pressure to afford **2** as a white solid (0.292 g, 0.854 mmol, 89%), mp 244 °C. The NMR spectra of compound **2** made by the two methods are identical.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(*tert*-butylcarbonyl)guanidine (**2a**)**. To the solution of the crude dione **9** (0.7 g, 2.5 mmol) in 25 mL of dry CHCl₃ was added *N*-isopropyl-*N''*-(*tert*-butylcarbonyl)guanidine (**11**) (0.5 g, 2.5 mmol). The reaction mixture was heated at 50 °C for 16 h. The reaction was quenched with water, extracted with CHCl₃, and dried over Na₂SO₄. The solvent was removed in vacuo and the crude product was purified with a silica gel column using 3% EtOAc/CHCl₃ as eluent to yield **2a** as a white solid (0.25 g, 22%), mp 227 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.55 (d, *J* = 2.4 Hz, 1H), 7.53(d, *J* = 8.6 Hz, 1H), 7.27 (dd, *J* = 8.6 and 2.4 Hz, 1H), 4.10 (m, 1H), 1.54 (s, 9H), 1.24 (d, *J* = 6.6 Hz, 6H). ¹³C NMR: δ 170.50, 168.40, 159.34, 156.09, 153.00, 132.51, 132.09, 131.34, 130.38, 128.62, 125.62, 85.59, 45.05, 29.70, 22.34. IR: 2979, 2930, 1766, 1729, 1532, 1474, 1142, 775, 734 cm⁻¹. MS (*m/z*): 444 (M⁺+1). Anal. (C₁₈H₂₁N₅O₄Cl₂) C, H, N.

An alternative method also has been successfully achieved by the following procedure: To the suspension of *N*-[1-(3,4-dichlorophenyl)-4,5-dioxo-4,5-dihydro-1*H*-imidazol-2-yl]-*N'*-isopropylguanidine (**2**) (27 mg, 0.079 mmol) in CHCl₃ (5 mL) was added first *p*-(dimethylamino)pyridine (DMAP, 0.964 mg, 0.0079 mmol), followed by the addition of di-*tert*-butyl dicarbonate (19 mg, 0.087 mmol)/CHCl₃ solution (10 mL), and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with water and extracted with CHCl₃ three times. The chloroform extracts were combined, dried over Na₂SO₄, and concentrated. The residue was purified by flash silica gel column chromatography and eluted with 10–30% diethyl ether in hexanes to furnish **2a** as crystals (11 mg, 31%). The NMR spectrum of the compound prepared by the alternative method is identical to the sample prepared by the condensation of compounds **9** and **11**.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(3-isobutylcarbonyl)guanidine (**2b**)**. To the solution of the crude dione **9** (0.7 g, 2.5 mmol) in 25 mL of dry CHCl₃ was added *N*-isopropyl-*N''*-(3-isobutylcarbonyl)guanidine (**12**) (0.5 g, 2.5 mmol). The reaction mixture was heated at 50 °C for 16 h. The reaction was quenched with water, extracted with CHCl₃, and dried over Na₂SO₄. The solvent was removed in vacuo and the crude product was purified with a silica gel column using 3% EtOAc/CHCl₃ as eluent to yield **2b** as a white solid (0.25 g, 22%), mp 227 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.55 (d, *J* = 2.4 Hz, 1H), 7.53(d, *J* = 8.6 Hz, 1H), 7.27 (dd, *J* = 8.6 and 2.4 Hz, 1H), 4.10 (m, 1H), 1.54 (s, 9H), 1.24 (d, *J* = 6.6 Hz, 6H). ¹³C NMR: δ 170.50, 168.40, 159.34, 156.09, 153.00, 132.51, 132.09, 131.34, 130.38, 128.62, 125.62, 85.59, 45.05, 29.70, 22.34. IR: 2979, 2930, 1766, 1729, 1532, 1474, 1142, 775, 734 cm⁻¹. MS (*m/z*): 444 (M⁺+1). Anal. (C₁₈H₂₁N₅O₄Cl₂) C, H, N.

dine (2b). Compound **2** (25 mg, 0.073 mmol) in 4 mL of dry CHCl_3 was added dry triethylamine (45.8 μL , 0.329 mmol), and the mixture was stirred at room temperature for 30 min. The suspension was cooled with an ice bath, and isobutyl chloroformate (38 μL , 0.292 mmol) was added dropwise with stirring. After the addition, the ice bath was removed and the reaction mixture was further stirred at room temperature for 2 h. The clear CHCl_3 solution was washed with H_2O three times, dried over Na_2SO_4 , and evaporated under reduced pressure to small volume. The crude product was collected and purified by silica gel flash chromatography (2% ethyl acetate/chloroform) to give the pure isobutyl derivative (**2b**) as a white solid (15 mg, 47% yield), mp 260 °C. ^1H NMR (600 MHz, CDCl_3): δ 12.44 (s, 1H), 7.55 (d, $J = 2.4$ Hz, 1H), 7.54 (d, $J = 8.6$ Hz, 1H), 7.28 (dd, $J = 8.6$ and 2.4 Hz, 1H), 4.12 (m, 1H), 4.02 (d, $J = 6.8$ Hz, 2H), 2.05 (m, 1H), 1.24 (d, $J = 6.6$ Hz, 6H), 0.99 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR: δ 170.73, 168.08, 159.18, 155.88, 154.10, 132.49, 132.10, 131.26, 130.35, 128.58, 125.6173.91, 45.15, 27.57, 22.20, 18.84. IR: 1769, 1723, 1624, 1546, 1472, 1387, 1308, 1242, 1205, 1059, 754 cm^{-1} . MS (m/z): 442 (M^+), 376, 316, 314, 272, 202. Anal. ($\text{C}_{18}\text{H}_{21}\text{Cl}_2\text{N}_5\text{O}_4$): C, H, N, Cl. The compound was identical to that prepared from the mixture of WR182393 in melting point and NMR.⁹

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(benzylcarbonyl)guanidine (2c).** To the solution of the crude dione **9** (0.10 g, 0.346 mmol) in 25 mL of dry CHCl_3 was added *N*-(benzylcarbonyl)-*N'*-isopropylguanidine (**10**) (0.122 g, 0.519 mmol) at room temperature. The reaction mixture was heated at 50 °C for 18 h and then quenched with water. The mixture was extracted with chloroform and dried over Na_2SO_4 . The CHCl_3 was removed in vacuo and the crude product was recrystallized from hexanes/ CHCl_3 to yield compound **2c** as light yellow crystals (51 mg, 0.11 mmol, 31%), mp 211 °C. ^1H NMR (300 MHz, CDCl_3): δ 7.54–7.51 (m, 2H), 7.41 (s, 5H), 7.27–7.23 (dd, $J = 8.6$ and 2.4 Hz, 1H), 5.25 (s, 2H), 4.12 (m, 1H), 2.46 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR: δ 170.83, 155.86, 153.87, 133.89, 132.56, 132.19, 131.22, 130.42, 129.21, 128.93, 128.65, 128.61, 125.60, 69.24, 45.25, 22.28. MS (m/z): 476 (M^+), 370, 316, 314, 236. Anal. ($\text{C}_{21}\text{H}_{19}\text{Cl}_2\text{N}_5\text{O}_4$) C, H, N, Cl.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(ethylcarbonyl)guanidine (2d).** Compound **2d** was prepared by two methods. Method A follows the same procedure for the preparation of **2b**, using pure compound **2** (0.788 g, 2.304 mmol) as starting material to yield compound **2d** as a white solid (0.632 g, 66%), mp 257 °C. ^1H NMR (CDCl_3 , 600 Hz) δ 7.56 (d, $J = 2.4$ Hz, 1H), 7.55 (d, $J = 8.3$ Hz, 1H), 7.29 (dd, $J = 2.4$ Hz, $J = 8.3$ Hz, 1H), 4.32 (q, $J = 7.1$ Hz, 2H), 4.16 (m, 1H), 1.39 (t, $J = 7.1$ Hz, 3H), 1.25 (d, $J = 6.6$ Hz, 6H). Anal. ($\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_4\text{Cl}_2$) C, H, N.

Method B adapted an alternative method for the preparation of **2c** as follows. To the solution of the crude dione **9** (0.413 g, 1.429 mmol) in 50 mL of dry CHCl_3 was added *N*-isopropyl-*N'*-(ethylcarbonyl)guanidine (0.206 g, 1.191 mmol). The reaction mixture was heated at 50 °C for 48 h. The reaction was quenched with water, extracted with chloroform, and dried over Na_2SO_4 . The solvent was removed in vacuo and the crude product was purified with a silica gel column using 3% EtOAc/ CHCl_3 as eluent to yield **2d** as a white solid (0.115 g, 20%). The melting point and NMR of compound **2d** made by both procedures and from the mixture WR182393⁹ are identical.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(1-hexylcarbonyl)guanidine (2e).** Compound **2e** was prepared by the same method for the preparation of **2a**, using pure compound **2** (0.800 g, 2.34 mmol) and hexyl chloroformate as starting materials to yield compound **2e** as a white color crystal (0.354 g, 32%). ^1H NMR (300 MHz, CDCl_3) δ 12.45 (s, 1H), 7.57–7.54 (m, 2H), 7.31–7.27 (dd, $J = 8.5$ and 2.4 Hz, 1H), 4.25 (t, $J = 6.8$ Hz, 2H), 4.12 (m, 1H), 1.74 (m, 2H), 1.35 (m, 6H), 1.25 (d, $J = 6.6$ Hz, 6H), 0.93 (t, $J = 6.4$ Hz, 3H). ^{13}C NMR: δ 170.78, 168.50, 159.20, 155.93, 154.15, 132.56, 132.18, 131.50, 130.41, 128.62, 125.61, 31.31, 28.38, 25.32, 22.49, 22.30, 14.00. MS (m/z): 470 (M^+), 418, 416, 368, 342, 314, 272, 230, 174.

***N*-[1-(3,4-Dichlorophenyl)-4,5-dioximidazolidin-2-ylidene]-*N'*-isopropyl-*N''*-(3-butenylcarbonyl)guanidine (2f).** Compound **2f** was prepared by the same method for the preparation of **2a**, using pure compound **2** (0.200 g, 585 mmol) and butenyl chloroformate as starting materials to yield compound **2f** as a white color solid (98 mg, 38%). ^1H NMR (300 MHz, CDCl_3) δ 12.46 (s, 1H), 9.22 (s, 1H), 7.56–7.53 (m, 2H), 7.30–7.26 (dd, $J = 8.6$ and 2.4 Hz, 1H), 5.85–5.72 (m, 1H), 5.20–5.13 (m, 2H), 4.29 (t, $J = 6.8$ Hz, 2H), 4.16 (m, 1H), 2.47 (q, $J = 6.8$ Hz, 2H), 1.24 (d, $J = 6.6$ Hz, 6H). ^{13}C NMR: δ 170.76, 168.08, 159.19, 155.88, 153.96, 132.63, 132.53, 131.26, 130.40, 128.61, 125.65, 118.32, 66.60, 45.23, 32.74, 22.27; MS (m/z): 440 (M^+), 388, 338, 269, 245, 180, 159. Anal. ($\text{C}_{18}\text{H}_{19}\text{Cl}_2\text{N}_5\text{O}_4 \cdot \text{H}_2\text{O}$) C, H, N.

Antimalarial Studies. (A) Sporozoite-Induced Test in Mouse.²³ The causal prophylactic antimalarial activity of the new carbamate derivatives were assessed in a sporozoite-challenged mouse model according to the following protocol, and the results are shown in Tables 1 and 2.

(1) Raising and Infecting the Mosquitoes. Cages of noninfected mosquitoes (*Anopheles stephensi*) are kept in a room maintained at 26.6 °C. They are allowed to feed on noninfected mice to obtain enough blood needed to produce eggs. Jars with wet cotton and moist paper towels are placed in the mosquito cages. Female mosquitoes lay their eggs on the moist paper towels. The eggs are collected and placed in enamel pans containing water. The eggs hatch and develop into larvae and then pupae. These stages are fed a liver powder suspension (2.5% liver powder in water) until the adults emerge. When the pupae have fully developed they are placed in empty jars, which are then placed in empty mosquito cages. After the adult mosquitoes emerge from the pupae stage the jars are removed. The cages containing mosquitoes to be infected are transferred to a room maintained at 21 °C.

Donor mice used to infect the mosquitoes are infected with 25,000 *P. yoelii* parasitized erythrocytes. The mosquitoes are allowed to feed on these malaria-infected mice on day 4 of their infection, when the parasitemia is low. The mice are anesthetized with Ketamine and placed on top of the mosquito cage, allowing the females to take an infected blood meal. The mosquitoes are maintained in this cool room for 17 days, when they are then taken for sporozoite isolation. During the last 4 days, a solution of PenStrep is fed to the mosquitoes to kill the bacteria in their gut. These mosquitoes are vacuumed into a plastic baggie that is heat-sealed. This bag of mosquitoes is placed on a freezing table to stun the mosquitoes. The bag is opened and the female mosquitoes are collected while the males are discarded. These infected females are ground with a mortar and pestle that has 0.1 mL of saline added. An additional 20 mL of saline is added to the mortar, and the suspension is filtered to remove large pieces of mosquitoes. The sporozoites in the saline suspension are then counted and diluted to get an inoculum of 250 000 sporozoites per 0.1 mL. This is then inoculated intraperitoneally into the test mice.

(2) Test Procedure. Mice are inoculated intraperitoneally with 250 000 sporozoites of *P. yoelii* on day 0. Blood examinations and weights are taken at frequent intervals, at least twice a week. The days of examination relative to day 0 vary to avoid weekend work. The test is run for a minimum of 31 days. All mice alive on the last day with negative blood films are considered cured. Mice losing >20% of their body weight at any time are sacrificed.

Nontreated, negative controls are run with every experiment to validate the infectivity of the sporozoites. While these sporozoites usually produce patent infections, a small number of mice remain blood negative. Caution must be taken when judging a compound as prophylactic when the patency rate in the negative controls is less than 100%.

Positive control groups are included occasionally, and they are treated with either primaquine or tafenoquine.

Routinely, 20 000 red blood cells (RBCs) are examined in a thin blood film before an animal is judged negative. The sensitivity of this technique is 0.01% infected RBCs. Each compound was ground with a mortar and pestle and suspended

Table 4. In Vitro Antimalarial Activity against Exoerythrocytic *P. yoelii* in Hepatocytes

compd	dose ($\mu\text{g/mL}$)	schizonts/well	means \pm SD	% inhibn
1	10	8, 9, 14	10 \pm 3.2	93
	1	100, 80, 87	89 \pm 4	40
1a	10	1, 1, 0	0.66 \pm 0.5	99
	1	48, 37, 53	46 \pm 8	69
1c	10	96, 85, 71	84 \pm 12.5	43
	1	106, 124, 103	111 \pm 11	25
1d	10	0, 2, 3	1.6 \pm 1.5	99
	1	60, 49, 53	54 \pm 5.5	63
2d	10	3, 2, 4	3 \pm 1	98
	1	130, 129, 140	133 \pm 6	10
2c	10	8, 27, 20	18 \pm 9.6	87
	1	147, 139, 128	138 \pm 9.5	6.7
WR182393	10			91
	1			50
tafenoquine	10	0, 1, 3	1.3 \pm 1.2	96
	1	29, 22, 27	26 \pm 2.9	39

in 0.5% hydroxyethylcellulose and 0.1% Tween 80 for compounds to be administered po, and those given sc were suspended in peanut oil. Each compound is prepared at three different dose levels.

Four-week-old male CD-1 mice, purchased from Charles River and weighing about 16–17 g were placed five per cage and allowed to acclimate for 4 days before being treated and then inoculated with sporozoites. They were fed food and water ad-lib and maintained at 24 °C with a 12 h light–dark cycle. The cages and water bottles were changed biweekly. The mice are weighed on days 0, 3, and 6 then biweekly when blood films were taken.

P. yoelii (17XNL) was used to infect mice that will be used to infect the mosquitoes. Compounds were administered once a day for three consecutive days, either po or sc, to mice on the day before (day –1), 4 h before (day 0), and the day after (day 1) inoculation of sporozoites intraperitoneally with 2.5×10^5 sporozoites of *P. yoelii* on Day 0. Whole-body weights were taken on day 0 and day 6 and then twice a week for 31 days. A blood film was taken on day 6 and then twice a week for 31 days. Mice losing greater than about 20% of their body weight were sacrificed. All mice alive on Day 31 with no parasites in a blood film were considered cured. A compound was considered active against either the sporozoite or the EE stage if no parasites were found in the blood films taken on day 6 or on subsequent blood films taken weekly for 31 days. A compound was considered to exhibit marginal activity if only low levels of parasites were found (less than about 10%) in blood films taken on day 6 or subsequently for 31 days. Mice alive on day 31 with no parasites found in any blood films were considered cured.

(B) In vitro Antimalarial Activity against *P. falciparum*. A number of the new compounds were assessed for in vitro antimalarial activity against blood stage parasite *P. falciparum* according to the method reported earlier.²⁴ The results are shown in Table 3.

(C) In vitro Mouse Liver Schizonticidal Assay. Mouse hepatocytes were obtained by in situ collagenase perfusion of mouse liver as described by Mellouk et al.²⁵ The prophylactic efficacy of the compounds was assessed in mouse hepatocyte cultures according to the method described by Loyevsky et al.²⁶ The results are shown in Table 4.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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