Antimalarial Activity of New Dihydroartemisinin Derivatives. 7. 4-(*p*-Substituted phenyl)-4(*R* or *S*)-[10(α or *β*)-dihydroartemisininoxy]butyric Acids[1−6]

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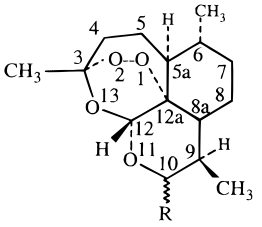
To search for water soluble dihydroartemisinin derivatives with higher efficacy and longer plasma half-life than artesunic or artelinic acid, a series of new stereoisomers of 4-(*p*-substituted phenyl)-4(*R* or *S*)-[10(α or *β*)-dihydroartemisininoxy]butyric acids were synthesized as new potential antimalarial agents. Two approaches were taken in the design of these new molecules in an attempt to (a) increase the lipophilicity of the molecule and (b) decrease the rate of oxidative dealkylation of the target compounds. The new compounds showed a 2−10-fold increase in *in vitro* antimalarial activity against D-6 and W-2 clones of *Plasmodium falciparum* than artemisinin or artelinic acid. *R*-diastereomers are, in general, more potent than the corresponding *S*-diastereomers. *p*-Chlorophenyl and *p*-bromophenyl derivatives showed *in vivo* oral antimalarial activity against *P. berghei* (with 3/8 cured) superior to that of artelinic acid (1/8 cured), whereas *p*-fluorophenyl and *p*-methoxyphenyl analogs demonstrated activity only comparable (1/8 cured) to that of artelinic acid at the same dosage level (64 mg/kg twice a day). The *in vivo* antimalarial activity of these new compounds correlates with their SD50 (50% parasitemia suppression dose). The biological results suggested that an electronic effect, besides the lipophylicity, may play a role in determining the efficacy of this class of compounds.

Background

The effectiveness of artemisinin (**1a**) and its derivatives as antimalarial drugs for the treatment of multidrug resistant *Plasmodium falciparum* has received increasing attention in recent years.[7−11] Due to the increasing prevalence of multidrug resistant *P. falciparum*, several artemisinin derivatives are being used increasingly in areas such as Southeast Asia.[11b] The practical values of these novel malarial therapeutic agents, nevertheless, are impaired by their (a) poor solubility in either oil or water,[8a] (b) high rate of parasite recrudescence after treatment,[8b] (c) short plasma half-life,[12,13] and/or (d) poor oral activity (active only at very high dosage).[8b] Chemical modifications of artemisinin has resulted in a number of analogs with improved efficacy and increased solubility in either oil, i.e., arteether and artemether[8a,14] (**1b** and **1c**), or water, i.e., sodium artelinate[15] (**1d**) and sodium artesunate[16] (**1e**). Both artemether and arteether are more potent than artemisinin but have a short plasma half-life and produce fatal CNS toxicity in chronically dosed rats and dogs.[17,18] Likewise, the usefulness of sodium artesunate in the treatment of cerebral malaria and multidrug resistant *P. falciparum* is offset by problems associated with its instability in aqueous solution,[1] the high rate of recrudescence, and the extremely short plasma half-life (20−30 min).[17a] Analogs with molecular modifications other than the 10-position of artemisinin or the simpler analogs of artemisinin were reported by several laboratories in recent years.[19] Some of the newly reported agents showed better *in vitro* antimalarial activities than aforementioned drugs of this class.

Sodium artelinate was designed to overcome the lability of sodium artesunate in aqueous solution. It is not only very stable in aqueous solution[1] but also has much longer plasma half-life (1.5−3 h) than oil soluble analogs.[17] It is highly effective when administered orally against *P. berghei*[20] and produced complete cures against *P. knowlesi* infection in rhesus monkeys at 15 mg/kg × 3 days.[21] Furthermore, recent CNS toxicity studies indicated that the water soluble dihydroartemisinin derivatives such as artesunate and artelinate possess substantially less CNS toxicity in rats and dogs than oil soluble analogs such as artemether and arteether.[18]



OIL SOLUBLE

1a R = =O ARTEMISININ

1b R = OCH_2CH_3 β-ARTEETHER

1c R = OCH_3 β-ARTEMETHER

WATER SOLUBLE

1d R = O-(C6H4)-COONa β-SODIUM ARTELINATE

1e R = $OCOCH_2CH_2COONa$ α-SODIUM ARTESUNATE

Pharmacokinetic data of arteether, artemether, and artesunic acid demonstrated a rapid enzymatic conversion of these compounds to dihydroartemisinin (DQHS) *in vivo* or in liver homogenate.[17] The high rate of recrudescence and the low rate of radical cure of dihydroartemisinin derivatives in general may be related to the short plasma half-life of the compounds, and the short plasma half-life, in turn, may be a result of fast conversion of DQHS derivatives to DQHS. The slower rate of dealkylation of artelinic acid to DQHS than artemether or arteether may account for the longer

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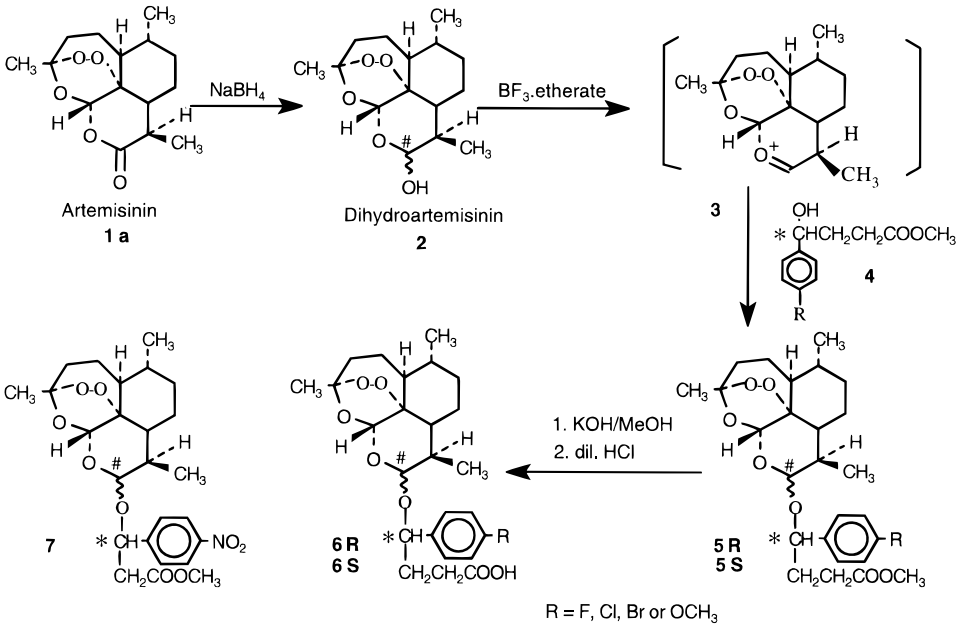
Scheme 1



plasma half-life of the former than the latter lipophilic compounds. A feasible approach to prolong the plasma half-life of this class of compounds, therefore, was to fabricate new analogs that are poor substrates for cytochrome P-450, an enzyme which catalyzes the oxidative hydroxylation of drugs *in vivo*. Theoretically, the substrate property (hydroxylation potential at the α-methylene carbon of artelinate) of the compounds can be altered by manipulating the electron density of the aromatic ring of artelinic acid or by exerting steric hindrance to the α-methylene carbon.[6] As part of an ongoing research project in search for stable, water soluble, high potency, long-acting, and orally active antimalarial drugs with minimum CNS toxicity, a series of diastereomers of 4-(*p*-substituted phenyl)-4(*R* or *S*)-[10(α or *β*)-dihydroartemisininoxy]butyric acids, based on the above mentioned rationale, were synthesized. The *in vitro* antimalarial activities of the new compounds were assessed against two clones (D-6 and W-2) of *P. falciparum*, and the *in vivo* activities of four water soluble final target compounds were determined in mice infected with *P. berghei* (KBG-173).

Chemistry

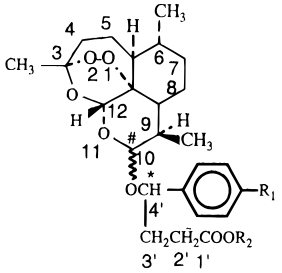
Dihydroartemisinin (**2**) was prepared by sodium borohydride reduction of artemisinin as previously reported.[1,2] The new ether derivatives of dihydroartemisinin were prepared by treatment of **2** with an appropriate alcohol **4** in the presence of boron trifluoride etherate at room temperature (Scheme 1). The yield of the purified condensation products **5** ranged from 40 to 75% (Table 1). The purification was achieved by the use of a silica gel chromatotron, preparative TLC, or column chromatography. In previous work related to this study,[6] the condensation between dihydroartemisinin and benzylic alcohols gave the *β*-isomers, which have small coupling constants (J = 3.5 Hz) between C_9-H and C_{10}-H, as the major condensation products. The α-isomers, which have a larger coupling constant (J = 9−10 Hz) between C_9-H and C_{10}-H than the *β*-isomers, were less than 5% of the condensation products. The relationship between coupling constant and the configuration at C_{10}-position was discussed in previous papers of this series.[1,2] An oxonium ion **3** was proposed to be involved in the ether formation.[1,2] Likewise, the condensation reaction between dihydroartemisinin (**2**) and the racemic mixture of the methyl 4-(*p*-substituted phenyl)-4-hydroxybutyrates (**4**) gave predominantly the *β*-isomers which consisted of equal amount of *R*- and *S*-diastereomers, except the methoxy analog whose product mixture contained mainly *β*(*R*)-isomer. No pure *β*(*S*)-isomer of methoxy analog [**5***β*(*S*), R = OMe] was isolated. The *R*-isomers of **5** formed fine crystals, but the *S*-isomers were generally low melting or gum. The differences in physical properties between *R*- and *S*-isomers render a higher isolated yield of the former than the latter.

Ether formation on reaction of dihydroartemisinin (**2**) with alcohols **4** yielded products **5** which added two new chiral centers. Therefore, a total of four stereoisomers in each product mixture is possible. Since preponderant condensation products are *β*-isomers, both *β*(*R*) and *β*(*S*) of fluoro, chloro, and bromo analogs were isolated and characterized. However, only one *β*(*R*)-isomer of methoxy analog was isolated; the corresponding *β*(*S*)-isomer was detected by NMR to be a minor component of the isomeric mixture. A small quantity of the pure α(*R*)-isomer of methoxy and chloro analogs were also obtained.

The starting alcohols (**4**) were prepared by esterification of the corresponding keto acids in boiling absolute methanol in the presence of boron trifluoride etherate. Sodium borohydride reduction of the keto esters gave the racemic alcohols **4**.

Although the resolution of a racemic mixture to its single *R*- and *S*-isomers is difficult and tedious, the multi-asymmetric carbon centers of the dihydroartemisinin moiety of the new analogs serve as an excellent internal resolving agent for separation of the diastereomeric derivatives. The dihydroartemisinin moiety, therefore, renders the new diastereomeric derivatives dissimilar in their physical properties such as melting

Table 1. Physical Properties of Dihydroartemisinin Derivatives



							^{1}H-NMR (ppm)			
compd	R_1	R_2	*(#)	formula	mp (°C)	yield (%)	C_{10}-H (d)	C_{12}-H (s)	C4′-H	analysis
5a	Cl	Me	*S*(β)	$C_{26}H_{35}O_7Cl$	gum	30	5.01 (J = 3.5 Hz)	4.95	4.77 (t, J = 5.8 Hz)	C,H
5b	Cl	Me	*R*(β)	$C_{26}H_{35}O_7Cl$	118	40	4.59 (J = 3.6 Hz)	5.47	4.83 (t, J = 6.8 Hz)	C,H,Cl
5c	Cl	Me	*R*(α)	$C_{26}H_{35}O_7Cl$	148	5	4.21 (J = 9 Hz)	5.19	4.89 (d,d J = 5.5, 9 Hz)	N/A
5d	F	Me	*R*(β)	$C_{26}H_{35}O_7F$	128	43	4.59 (J = 3.5 Hz)	5.48	4.82 (t, J = 6.6 Hz)	C,H
5e	F	Me	*S*(β)	$C_{26}H_{35}O_7F$	gum	30	5.01 (J = 3.5 Hz)	4.90	4.74 (t, J = 6 Hz)	C,H
5f	Br	Me	*R*(β)	$C_{26}H_{35}O_7Br$	123	55	4.60 (J = 3.4 Hz)	5.47	4.81 (t, J = 7 Hz)	C,H,Br
5g	Br	Me	*S*(β)	$C_{26}H_{35}O_7Br$	gum	20	5.00 (J = 3.3 Hz)	4.95	4.75 (t, J = 5.9 Hz)	C,H,Br
5h	OMe	Me	*R*(β)	$C_{27}H_{38}O_8$	131	45	4.62 (J = 3.5 Hz)	5.49	4.77 (t, J = 6.7 Hz)	C,H
5i	OMe	Me	*R*(α)	$C_{27}H_{38}O_8$	124.6	7	4.23 (J = 9 Hz)	5.18	4.85 (d,d, J = 5.7, 9 Hz)	C,H
6a	Cl	H	*S*(β)	$C_{25}H_{33}O_7Cl$	gum	85	5.03 (J = 3.4 Hz)	4.94	4.79 (t, J = 6 Hz)	C,H,Cl
6b	Cl	H	*R*(β)	$C_{25}H_{33}O_7Cl$	147	75	4.61 (J = 3.6 Hz)	5.48	4.85 (t, J = 6.5 Hz)	C,H,Cl
6d	F	H	*R*(β)	$C_{25}H_{33}O_7F$	108	70	4.61 (J = 3.4Hz)	5.49	4.85 (t, J = 6.5 Hz)	C,H
6e	F	H	*S*(β)	$C_{25}H_{33}O_7F$	gum	85	5.04 (J = 3.4 Hz)	4.89	4.76 (t, J = 6 Hz)	C,H
6f	Br	H	*R*(β)	$C_{25}H_{33}O_7Br$	145	70	4.60 (J = 3.5 Hz)	5.48	4.84 (t, J = 6.7 Hz)	C,H,Br
6g	Br	H	*S*(β)	$C_{25}H_{33}O_7Br$	gum	75	5.03 (J = 3.2 Hz)	4.95	4.79 (t, J = 5.9 Hz)	C,H,Br[a]
6h	OMe	H	*R*(β)	$C_{26}H_{36}O_8$	70	70	4.63 (J = 3.5 Hz)	5.50	4.80 (t, J = 6.6 Hz)	C,H

[a] Calcd for Br = 15.24, found 14.32.

point, solubility, and R_f values on TLC. Thus, the diastereomers of compounds **5** were successfully separated by the use of a normal phase silica gel chromatotron, preparative TLC, or column chromatography. The hydrolysis of **5** with 2.5% KOH/MeOH gave the corresponding carboxylic potassium salt which was converted to the free carboxylic acids by addition of dilute HCl to give **6**. The identities of all products were established by ^{1}H-NMR (Table 1) spectrometry and elemental analysis.

The proton NMR spectra of *R*- and *S*-diastereomers of **5** and **6** displayed large chemical shift differences (Δ ppm) from signals of C_{10}-H and C_{12}-H of the dihydroartemisinin moiety as were observed previously for this type of compounds. The proton signals of C_{10}-H from *R*-isomers resonate 0.4−0.5 ppm upfield from those of the corresponding *S*-isomers. On the contrary, the proton signals from C_{12}-H of *R*-diastereomers are 0.4−0.6 ppm downfield from those of *S*-diastereomers. The substantial differences in chemical shifts observed between *R*- and *S*-diastereomers of the related analogs **7** were found, by X-ray crystallographic data, to be due to conformational differences in both diastereomers.[22] The aromatic ring of the *R*-isomer of compound **7** is positioned over the C_{10}-H and away from C_{12}-H. On the other hand, the benzene ring of the *S*-isomer is positioned over C_{12}-H and away from C_{10}-H. The diamagnetic anisotropic effects of the benzene ring, therefore, shielded the C_{10}-H in the *R*-isomer and C_{12}-H in the *S*-isomer which lead to the observed differences in their chemical shifts. It is interesting to note that the benzene ring of these new compounds also exerts a shielding effect to the C_9-CH_3 protons of the R-isomers, causing an increase in chemical shift differences between *R*- and *S*-isomers from less than 0.05 ppm to 0.15 ppm.

Table 2. *In Vitro* Antimalarial Activity against *P. falciparum*

	IC_{50} (ng/mL)			IC_{50} (ng/mL)	
compd	W-2	D-6	compd	W-2	D-6
artemisinin	2.138	3.912	**5h**	0.6468	0.8266
artelinic acid	1.380	4.070	**6a**	1.261	2.403
5a	0.7940	0.903	**6b**	0.357	0.380
5b	0.6290	0.7275	**6d**	1.339	2.210
5c	0.6560	0.3910	**6e**	0.828	2.332
5d	0.3980	0.4305	**6f**	0.313	0.405
5e	0.4878	0.6158	**6g**	0.419	1.359
5f	0.5433	0.6727	**6h**	3.452	3.816
5g	0.6200	1.0095			

Results and Discussion

The new dihydroartemisinin derivatives were tested *in vitro* against two clones of human malaria, *P. falciparum* D-6 (Sierra Leone I clone) and W-2 (Indochina clone). The former clone is a strain that is resistant to mefloquine and the latter to chloroquine, pyrimethamine, sulfadoxine, and quinine. As shown in Table 2, all new compounds demonstrated activity 2−10-fold better than that of artelinic acid or artemisinin against both clones. The esters **5a**−**h** and three of the free acid analogs **6b, 6f**, and **6g** with IC_{50} values less than 1 ng/mL are as active as the lipophilic arteether. It was observed previously in this laboratory that conversion of esters to free carboxylic acids of compounds with smaller molecular weight than those in this study, such as methyl artelinate and related compounds, reduced antimalarial activity severalfold. This observation was used as a basis in this study to design compounds with higher lipophilicity than artelinic acid by increasing the length of the aliphatic acid side chain which compensates for the loss of lipophilicity on conversion of the ester to acid. The antimalarial results indicated that both esters (**5b** and **5f**) and acids (**6b** and **6f**) of Cl and Br analogs of the new compounds

Table 3. *In Vivo* Antimalarial Activity against *Plasmodium berghei* and the Percent Suppression of Parasitemia

compd	dose (mg/kg)	T/C[a]	no. of cures (C)	SD50 (mg/kg)	SD90 (mg/kg)
control	0	1.0	0/8		
1d				56.0	n/a
	16	2.7	0/8 (C),[b] 7/8 (A)[c]		
	64	4.3	1/8 (C), 7/8 (A)		
6d				28.16	58.07
	4	1.5	0/8 (C), 1/8 (A)		
	16	2.8	1/8 (C), 6/8 (A)		
	64	3.3	1/8 (C), 7/8 (A)		
6b				14.39	45.04
	4	2.3	0/7 (C), 4/7 (A)		
	16	3.8	1/8 (C), 7/8 (A)		
	64	4.8	3/8 (C), 5/8 (A)		
6f				16.26	48.18
	4	2.7	0/8 (C), 7/8 (A)		
	16	3.5	0/8 (C), 7/8 (A)		
	64	5.8	3/8 (C), 5/8 (A)		
6h				25.06	54.99
	4	1.7	0/8 (C), 3/8 (A)		
	16	2.5	0/8 (C), 8/8 (A)		
	64	4.0	1/8 (C), 7/8 (A)		

[a] T/C = life span of treated mice/control. [b] C = cure. [c] A = active. The terms cure and active are defined in the Experimental Section.

displayed similar *in vitro* activity against *P. falciparum*. This is in line with the intended design to obtain an optimal lipophilicity of the final target carboxylic acid for better antimalarial activity. The lipophilicity of **6b** (Cl) is comparable to that of **6f** (Br) and is higher than that of artelinic acid or artemether as evidenced by the longer retention time of the former than the latter in reverse phase high-pressure liquid chromatography. The *in vitro* antimalarial results indicated that most of these new compounds were slightly more active against W-2 (chloroquine-resistant clone) than against D-6 (mefloquine-resistant clone), which is in consistent with the previous observations on related compounds of this class.[1−6]

Since the main purpose of this study is to fabricate new water soluble, stable, and long-acting dihydroartemisinin derivatives with minimum CNS toxicity and the water soluble analogs are less toxic and more active orally than the oil soluble esters, only four of the water soluble final target compounds 6β(*R*) (**6b**, **6d**, **6f**, and **6h**) were selected for *in vivo* studies to determine their oral activity against *P. berghei*.. Each of these compounds cleared parasitemia rapidly and produced SD50s and SD90s better than artelinic acid (Table 3) and showed oral antimalarial activity at dosage of 64 mg/kg given twice daily for 3 days. However, only Cl (**6b**) and Br (**6f**) analogs exhibit better *in vivo* antimalarial activity (3/8 cured) than artelinic acid (1/8 cured) at higher dosage. Fluoro (strong electron-withdrawing group) and methoxy (strong electron-donating function) analogs are only equally active (1/8 cured) as that of artelinic acid at the same dosing schedule. The *in vivo* antimalarial activity correlates well with the *in vitro* results since the Cl and Br analogs are also more active than the corresponding F and MeO analogs in *in vitro* test. As steric hindrance and lipophilicity are similar for all four compounds, the results suggested that the electronic effect may have played an important role in manipulating the efficacy of this class of compounds. Additional pharmacokinetic studies of these compounds are currently in progress to determine whether the higher efficacy of Cl (**6b**) and Br (**6f**) analogs than F (**6d**) and MeO (**6h**) analogs are related to the longer plasma half-life and whether the longer plasma half-life of the compounds are a result of slower rate of oxidative dealkylation of the side chain. The pharmacokinetic data may assist in further design of better and longer acting water soluble derivatives which are devoid of CNS toxicity.

Experimental Section

Chemistry. All melting points were determined on a Mettler FP 62 melting point apparatus. Infrared spectra were obtained on a Nicolet 20SXB FT-IR spectrometer. NMR spectra were determined on a Brucker AC300 spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and the results were within 0.4% of the theoretical values, except where noted.

Condensation of Dihydroartemisinin (2) with Alcohols 4. Dihydroartemisinin[1] (**2**, 0.5 g, 1.8 mmol) was dissolved in 70 mL of anhydrous Et_2O. To the solution was added 3 mmol (excess) of an appropriate alcohol, followed by 0.25 mL of $BF_3{\cdot}Et_2O$. The reaction mixture was stirred at room temperature for 24 h, washed successively with 5% aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The resultant crude products were purified with a silica gel chromatotron, preparative TLC, or silica gel column chromatography using EtOAc/hexane or petroleum ether as eluant to give the pure single *R*- or *S*-diastereomer. The physical properties of these compounds and their ^{1}H-NMR data are listed in Table 1.

Conversion of Esters 5 to the Corresponding Carboxylic Acids 6. Ester **5** (1 g) was dissolved in 30 mL of 2.5% KOH/MeOH and allowed to stand at room temperature for 2 days. To the solution was added an equal volume of H_2O (30 mL), and the MeOH was removed under reduced pressure. Upon being cooled in an ice bath, the aqueous solution was acidified with dilute HCl and extracted three times with Et_2O. The ether extracts were combined, dried over Na_2SO_4, and evaporated to dryness to give crude products which were recrystallized from appropriate solvents. The physical properties of these new acids and their ^{1}H-NMR data are listed in Table 1.

Biology

(a) *In Vitro* Antimalarial Studies. The *in vitro* assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[23] and Chulay et al.[24] Two *P. falciparum* malaria parasite clones, from CDC Indochina III (W-2) and CDC Sierra Leone I (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates.[25] The W-2 clone is susceptible to mefloquine but resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the D-6 clone is naturally resistant to mefloquine but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine. Test compounds were initially dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium supplemented with 25 mM Hepes, 32 mM $NaHCO_3$, and 10% Albumax I (Gibco BRL, Grand Island, NY). These solutions were subsequently serially diluted 2-fold with a Biomek 1000 (Beckman, Fullerton, CA) over 11 different concentrations. The parasites were exposed to serial dilutions of each compound for 48 h and incubated at 37 °C with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of [^{3}H]hypoxanthine. After a further incubation of 18 h, parasite DNA was harvested from each microtiter well using Packard Filtermate 196 Harvester (Meriden, CT) onto glass filters. Uptake of [^{3}H]hypoxanthine was measured with a Packard topcount scintillation counter. Concentration−response data were analyzed by a nonlinear regression logistic dose response model, and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated (Table 2).

(b) *In Vivo* Antimalarial Studies. The *in vivo* efficacy of the artelinic acid analogs were determined in a modified Thompson test. This test measures the survivability of mice

and parasitemia clearance following administration of drug on days 3−5 postinfection. In brief, 5×10^6 *P. berghei*-infected erythrocytes (KBG-173 strain) were inoculated intraperitoneally to female mice that weighed 24−30 g. By day three postinfection, parasitemia ranged from 1.0 to 3.7%. Each drug, dissolved in 5% sodium bicarbonate, was administered po twice daily from day 3 to day 5 postinfection. Total doses for each drug ranged from 1.5 to 384 mg/kg with eight mice included in each dosage group. The percent suppression of parasitemia in the treated mice compared to the untreated controls was determined for each test compound; the SD50 (50% suppression dose) and SD90 were determined from a nonlinear regression logistic dose response fit at day 6 parasitemia data. Survival of mice to day 60 postinfection was considered a cure (C). Compounds were considered active (A) when the survival time of the treated mice was greater than twice the control mice, i.e., 12−14 day.

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