Published in final edited form as:
Bioorg Med Chem Lett. 2014 June 1; 24(11): 2440–2443. doi:10.1016/j.bmcl.2014.04.025.

Antimalarial Chemotherapy: Artemisinin-Derived Dimer Carbonates and Thiocarbonates

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Abstract

Several 2-carbon-linked trioxane dimer secondary alcohol carbonates **14** and thiocarbonates **15**, combined with mefloquine and administered in a low single oral dose, prolonged the survival times of malaria-infected mice much more effectively than the popular monomeric antimalarial drug artemether plus mefloquine. Three dimer carbonates **14** and one dimer thiocarbonate **15** partially cured malaria-infected mice.

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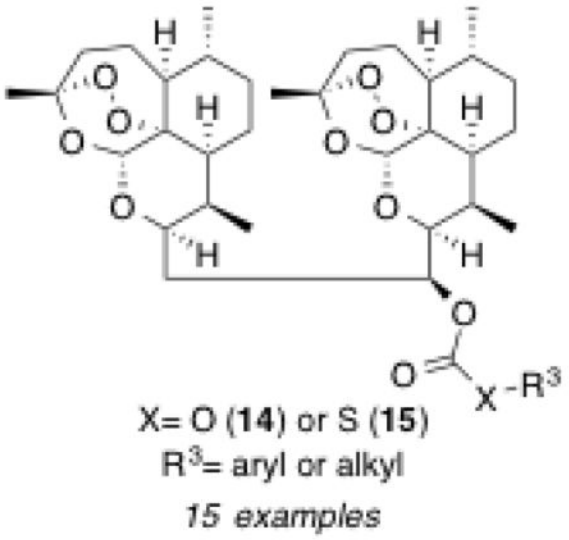
Supplementary data

Supplementary data (experimental and tabular spectral data) associated with this article can be found in the online version.



Keywords

Antimalarial chemotherapy; Trioxane dimers; Single oral dose ACT; Oral bioavailability

In 2013, a total of 107 countries reported malaria as an ongoing epidemic exposing an estimated 3.4 billion people to the *Plasmdium falciparum* malaria parasites.[1] Nearly one million people, mostly children, die each year from infection with malaria.[2–4] While ongoing efforts have been made toward the development of a fully prophylactic malaria vaccine, only partial success has been reported.[5,6] The efficacy of antimalarial chemotherapy using standard drugs like chloroquine is being severely compromised by widespread parasite resistance.[7,8] Therefore, the discovery of a new class of peroxide-containing antimalarials such as artemisinin (**1**)[9] and its first generation derivatives artemether (**2**) and sodium artesunate (**3**) has led to their widespread use (Figure 1). Indeed, the World Health Organization (WHO) now recommends artemisinin combination therapy (ACT) as standard operating procedure, combining a fast-acting but short-lived trioxane with a long-lasting adjuvant.[10] Current examples of combinations include artemether (**2**) plus lumefantrine (**4**),[11] artesunate (**3**) plus mefloquine (**5**), and artesunate (**3**) plus pyronaridine (**6**).[12] An ideal regimen for curing infected people is a single low oral dose of ACT. Toward this goal, others[13–18] and we[19–22] have prepared several artemisinin derivatives that cure malaria-infected mice.

Guided by structure-activity relationships (SAR), ongoing efforts have been made toward improving the oral bioavailability and minimizing the metabolic shortcomings of artemisinin and its first generation derivatives.[23] Tethering two artemisinin units together through the C10 position forms a C10 non-acetal dimeric trioxane structure, which has proven often to be more antimalarially potent than its corresponding monomeric counterpart.[24–26] We have highlighted the high efficacy of a low single oral dose ACT using new artemisinin-derived trioxane dimers with linkers of different length: 5-carbon (**7**),[27] 4-carbon (**8**),[28] 3-carbon (**9**),[29,30] and 2-carbon (**10**, Figure 2). Two-carbon-linked trioxane dimer ketone **10** (prepared

from artemisinin in 36% overall yield) and especially some of its oxime NH-aryl carbamates **11** are effective antimalarials,[31] as are 2-carbon-linked dimer secondary alcohols **12a** and **12b**; using only a single low oral dose of several NH-aryl carbamate derivatives **13** combined with mefloquine hydrochloride substantially prolonged the survival times of malaria-infected mice (Scheme 1).[31,32]

Encouraged by our recent results,[31,32] we prepared a novel series of 2-carbon-linked dimer carbonates **14a–l** and thiocarbonates **15a–c** (Scheme 2). The log P values for all of these orally bioavailable dimer carbonates **14a–l** and thiocarbonate **15a–c** range between 7.3–9.3.[33] The log P value of parent secondary alcohol **12b** is 6.0.[33] The log P of artemether (**2**) is 3.5.[33] Facile conversion of parent secondary alcohol **12b** was accomplished in one step from commercially available chloroformates and thiochloroformates, producing fifteen carbonates **14** and thiocarbonates **15** in moderate to high yields. In such cases where the purified product yield was less than 50% (**14b** and **14f**), starting dimer alcohol was recovered. All carbonates **14** and thiocarbonates **15** were purified by chromatography on silica gel and their purity (> 95%) was established through normal phase HPLC analysis using an isocratic mobile phase (20% EtOAc in hexane).

For preclinical drug development, *in vivo* efficacy data in mice (as shown in this manuscript) are more valuable and more stringent than *in vitro* potency data. Our experience with trioxanes over the past two decades supports the generalization that, within a family of antimalarially potent trioxanes, *in vitro* potency (IC-50) data often do not accurately predict *in vivo* efficacy levels. Therefore, we chose to evaluate our new antimalarial trioxane dimers **14** and **15** directly by oral administration *in vivo*. Stock solutions were prepared by dissolving mefloquine hydrochloride (2.16 mg) in 113 μL of 7:3 Tween 80:ethanol. Then 0.72 mg of either a dimer carbonate **14** or a dimer thiocarbonate **15** was added. After approximately 18 hours at room temperature, 1067 μL of deionized water was added, and then 200 μL of this stock solution was administered by oral gavage one day post infection to 5-week old C57BL/6J male mice (from Jackson Laboratory) that weighed approximately 20 g, which had been infected with *P. berghei* ANKA strain (2×10^7 parasitized erythrocytes). Each mouse (four mice per group) was treated orally with a single 200 μL dose of stock solution, corresponding to a dose of 6 mg/kg of trioxane dimer in combination with 18 mg/kg of mefloquine hydrochloride. As expected, all 2-carbon-linked dimer carbonates **14** and thiocarbonates **15** produced antimalarial chemotherapeutic results. The mouse survival data are shown in Table 1.

Parasitemia levels were evaluated on day 3 after infection and showed >99.9% suppression in all trioxane dimer-treated mice, indicating very rapid and high antimalarial activity. In contrast, the control mice (infected but no drug) had an average of 10% (9, 10, 11, 10) parasitemia on day 3 after infection, which resulted in their death on an average of 7 days (6, 7, 7, 8) after infection. All trioxane dimers **14** and **15** (with the exception of the two carbonates **14a** and **14f**) displayed average survival times longer than that of the antimalarial drug artemether (**2**, 21.5 day average survival). Mefloquine hydrochloride alone (18 mg/kg) prolonged mouse survival until only day 18. Fluoroethyl carbonate **14c** (26 days), chloroethyl carbonate **14e** (28 days), and chlorophenyl carbonate **14l** (26 days) had average survival times notably longer than that of artemether (**2**, 21.5 days). Most significantly, 2-

carbon-linked dimer thiocarbonate **15b** prolonged survival of all four of the malaria-infected mice till at least day 30 using only one 6 mg/kg oral dose of this trioxane dimer. Mouse survival until day 30 with no parasitemia detected is a widely accepted measure of a drug's antimalarial efficacy and indicates a complete cure. Day 30 survival with no parasitemia and with normal behavior and appearance was achieved in one of four mice treated with fluoroethyl carbonate dimer **14c**, with chloroethyl carbonate dimer **14e**, and with chlorophenyl carbonate dimer **14l**. All four mice treated with thiocarbonate dimer **15b** survived till at least day 30, at which time one of the four mice in this group had no detectable parasitemia and behaved normally and looked healthy. As determined by analytical thin layer chromatography, thiocarbonate **15b** is stable at pH 2 for at least 24 hours at 37 °C. Several sulfur-containing antimalarial trioxanes have been reported recently.[13,21,22,27,29,34]

In conclusion, it is noteworthy that the partially curative carbonates **14c**, **14e**, **14l** and thiocarbonate **15b** all had average survival times (26–30 days) which were considerably higher than the average survival times of their parent dimer secondary alcohol **12b** (19.5 days). This preclinical drug development result suggests that these orally bioavailable dimer carbonates and thiocarbonates act as new chemical entities and possibly as prodrugs[35] of the parent dimer alcohol **12b**. Dimer secondary alcohol **12b** represents a versatile platform for preparation of other derivatives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank NIH (Grant R37 AI 34885 to G.H.P.), The Johns Hopkins Malaria Research Institute, and the Bloomberg Family Foundation for financial support.

Abbreviations

SAR	structure-activity relationship
ACT	artemisinin combination therapy
HPLC	high performance liquid chromatography
DMAP	4-dimethylaminopyridine
Py	pyridine

References and notes

1. World Malaria Report 2013. World Health Organization; Geneva Switzerland: 2013. (http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/index.html [accessed 28 January 2014]
2. Murray CJL, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Hqqaring D, Fullman N, Naghavi M, Lozono R, Lopez AD. Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet. 2012; 379:413–431. [PubMed: 22305225]
3. Gulland A. Brit Med J. 2012; 344:895.

4. Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, Winzeler EA, Sinden RE, Leroy D. PLoS Med. 2012; 9:e1001169.10.1371/journal.pmed.1001169 [PubMed: 22363211]
5. Schwartz L, Brown GV, Genton B, Moorthy VS. Malaria Journal. 2012; 11:1–22. [PubMed: 22212246]
6. Thera MA, Plowe CV. Annu Rev Med. 2012; 63:345–357. [PubMed: 22077719]
7. Olliaro, PL.; Boland, PB. Clinical Public Health Implications of Antimalarial Drug Resistance. In: Rosenthal, PJ., editor. Antimalarial Chemotherapy: Mechanisms of Action, Resistance and New Directions in Drug Discovery. Humana Press; Totowa, NJ: 2001. p. 65-84.
8. Schlitzer M. ChemMedChem. 2007; 2:944–986. [PubMed: 17530725]
9. Miller LH, Su X. Cell. 2011; 146:855–853. [PubMed: 21907397]
10. Guidelines for Treatment of Malaria. World Health Organization; Geneva, Switzerland: 2006.
11. Welcome to Coartem.com the Novartis Malaria Initiative Website. Coartem. [accessed January 30, 2014] http://http://www.coartem.us.com/info/coartem/what-is-coartem.jsp
12. MMV. [accessed January 30, 2014] Pyramax (Pyronaridine-Artesunate). http://http://www.mmv.org/research-development/rd-portfolio
13. Haynes RK, Fugmann B, Stetter J, Rieckmann K, Hans-Dietrich H, Chan H–W, Cheug M–K, Lam W–L, Wong H–N, Croft SL, Vivas L, Rattry L, Stewart L, Peters W, Robinson BL, Edstein MD, Kotecka B, Kyle DE, Beckermann B, Gerisch M, Radtke M, Schmuck G, Steink W, Wollborn U, Schmeer K, Romer A. Angew Chem, Int Ed. 2006; 45:2082.
14. Pacorel B, Leung SC, Stachulski AV, Davis J, Viva L, Lander H, Ward SA, Kaiser M, Brun R, O'Neill PM. J Med Chem. 2010; 53:633. [PubMed: 19957999]
15. Chadwick J, Jones M, Mercer AE, Stock PA, Ward SA, Park BK, O'Neill PM. Bioorg Med Chem. 2010; 18:2586. [PubMed: 20227283]
16. Jung M, Lee K, Kendrick H, Robinson BL, Croft SL. J Med Chem. 2002; 45:4940. [PubMed: 12383020]
17. See also: Wang X, Dong Y, Wittlin S, Charman SA, Chiu FCK, Chollet J, Katneni K, Mannila J, Morizzi J, Ryan E, Scheurer C, Steuten J, Tomas JS, Snyder C, Vennerstrom JL. J Med Chem. 2013; 56:2547. [PubMed: 23489135]
18. Winter RW, Kelly JX, Smilkstein MJ, Dodean R, Bagby GC, Rathbun RK, Levin JI, Hinrichs D, Riscoe MK. Experimental Parasitology. 2006; 114:47. [PubMed: 16828746]
19. Posner GH, Ploypradith P, Parker MH, O'Dowd H, Woo S-H, Northrop J, Krasavin M, Dolan P, Kensler TW, Xie S, Shapiro TA. J Med Chem. 1999; 42:4275. [PubMed: 10543871]
20. Moon DK, Sighal V, Kumar N, Shapiro TA, Posner GH. Drug Development Research. 2010; 71:76. [PubMed: 20686674]
21. Slack RD, Mott BT, Woodard LE, Tripathi A, Sullivan D, Nenortas E, Girdwood SCT, Shapiro TA, Posner GH. J Med Chem. 2012; 55:291. [PubMed: 22128829]
22. Jacobine AM, Mazzone JR, Slack RD, Tripathi AK, Sullivan DJ, Posner GH. J Med Chem. 2012; 55:7892. [PubMed: 22891714]
23. Slack RD, Jacobine AM, Posner GH. Med Chem Commun. 2012:3.
24. Woerdenbag HJ, Moskal TA, Pras N, Malingre TM, Elferaly FS, Kampinga HH, Konings AWT. J Nat Prod. 1993; 56:849. [PubMed: 8350087]
25. Posner GH, Chang W, Hess L, Woodard L, Sinishtaj S, Usera AR, Maio W, Rosenthal AS, Kalinda AS, D'Angelo JG, Petersen KS, Stohler R, Chollet J, Santo-Tomas J, Snyder C, Rottmann M, Wittlin S, Brun R, Shapiro TA. J Med Chem. 2008; 51:1035. [PubMed: 18232653]
26. Chadwick J, Mercer AE, Park BK, Cosstick R, O'Neill PM. Bioorg Med Chem. 2009; 17:1325. [PubMed: 19136263]
27. Moon DK, Tripathi A, Sullivan D, Siegler MA, Parkin S, Posner GH. Bioorg Med Chem Lett. 2011; 21:2773. [PubMed: 20952197]
28. Paik IH, Xie S, Shapiro TA, Labonte T, Narducci-Sarjeant AA, Baege AC, Posner GH. J Med Chem. 2006; 49:2731. [PubMed: 16640333]
29. Rosenthal AS, Chen X, Liu JO, West DC, Hergenrother PJ, Shapiro TA, Posner GH. J Med Chem. 2009; 52:1198. [PubMed: 19186946]

30. Woodard LE, Chang W, Chen X, Lui JO, Shapiro TA, Posner GH. J Med Chem. 2009; 52:7458. [PubMed: 19586052]

31. Mott BT, Tripathi A, Siegler MA, Moore CD, Sullivan DJ, Posner GH. J Med Chem. 2013; 56:2630–2641. [PubMed: 23425037]

32. Conyers RC, Mazzone JR, Siegler MA, Tripathi AK, Sullivan DJ, Mott BT, Posner GH. Bioorg Med Chem Lett. 2014; 24:1285. [PubMed: 24508128]

33. Log P values for artemether (2), parent secondary dimer alcohol 12b, and 2C-linked trioxane dimers 14a–l, and 15a–c were calculated using MarvinSketch version 5.12.3.

34. Waknine-Grinberg JH, Hunt N, Bentura-Marciano A, McQuillan JA, Chan H–W, Chan WC, Barenholz Y, Haynes RK, Golenser J. Malaria Journal. 2010; 9

35. Wolfe AL, Duncan KK, Parelkar NK, Weir SJ, Vielhauer GA, Boger DLJ. Med Chem. 2012; 55:5878.

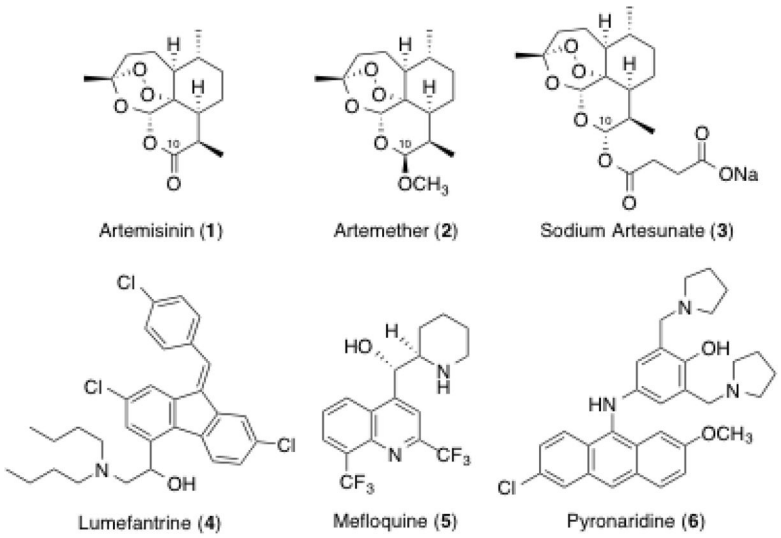


Figure 1.

Artemisinin (**1**), first generation derivatives (**2** & **3**), and adjuvant therapeutic drugs used in ACT (**4**–**6**).

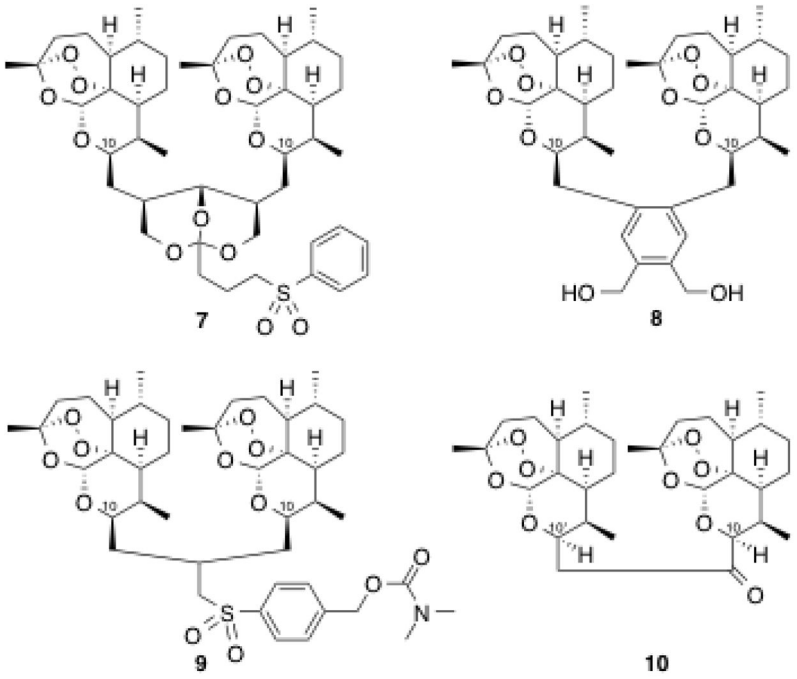
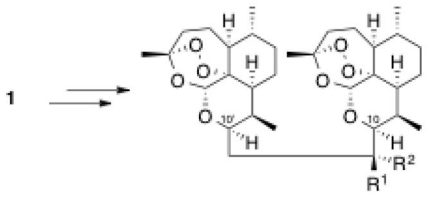


Figure 2.
Representative 5-, 4-, 3-, and 2-carbon-linked dimer trioxanes

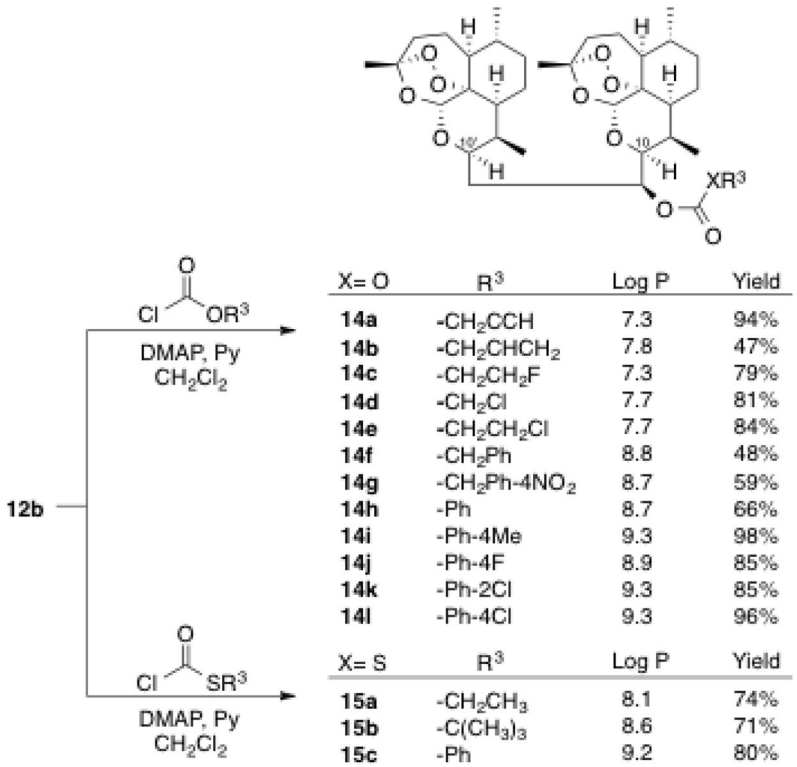


10 $R^1 = R^2 = O$ (ref. 31)
11 $R^1 = R^2 = NOC(O)NHAr$ (ref. 31)

12a $R^1 = H$, $R^2 = OH$ (ref. 32)
12b $R^1 = OH$, $R^2 = H$ (ref. 32)
13 $R^1 = OC(O)NHAr$, $R^2 = H$ (ref. 32)

14 $R^1 = OC(O)OR^3$, $R^2 = H$ (this work)
15 $R^1 = OC(O)SR^3$, $R^2 = H$ (this work)

Scheme 1.
Two-carbon-linked dimer derivatives



X= O	R^3	Log P	Yield
14a	$-CH_2CCH$	7.3	94%
14b	$-CH_2CHCH_2$	7.8	47%
14c	$-CH_2CH_2F$	7.3	79%
14d	$-CH_2Cl$	7.7	81%
14e	$-CH_2CH_2Cl$	7.7	84%
14f	$-CH_2Ph$	8.8	48%
14g	$-CH_2Ph-4NO_2$	8.7	59%
14h	-Ph	8.7	66%
14i	-Ph-4Me	9.3	98%
14j	-Ph-4F	8.9	85%
14k	-Ph-2Cl	9.3	85%
14l	-Ph-4Cl	9.3	96%

X= S	R^3	Log P	Yield
15a	$-CH_2CH_3$	8.1	74%
15b	$-C(CH_3)_3$	8.6	71%
15c	-Ph	9.2	80%

Scheme 2.

Two-carbon-linked dimer carbonates **14a–l** and thiocarbonates **15a–c**

Table 1

In vivo antimalarial efficacy using a single oral dose of trioxane dimer (6 mg/kg) combined with mefloquine hydrochloride (18 mg/kg) in *P. berghei* ANKA-infected mice

trioxane	survival after infection (days)	avg survival[a]	% parasitemia suppression[b]
12b	14, 20, 21, 23	19.5	>99.9
14a	14, 15, 15, 30	18.5	>99.9
14b	13, 23, 30, 30	24	>99.9
14c	30, 21, 21, 30	**25.5**	>99.9
14d	23, 21, 23, 30	24.3	>99.9
14e	30, 21, 30, 30	**27.8**	>99.9
14f	21, 14, 15, 20	17.5	>99.9
14g	23, 21, 21, 30	23.8	>99.9
14h	21, 20, 23, 30	23.5	>99.9
14i	30, 23, 14, 30	24.3	>99.9
14j	27, 20, 21, 30	24.5	>99.9
14k	30, 20, 14, 23	21.8	>99.9
14l	30, 30, 30, 15	**26.3**	>99.9
15a	23, 20, 21, 29	23.3	>99.9
15b	30, 30, 30, 30	**30**	>99.9
15c	15, 23, 30, 30	24.5	>99.9
controls:			
vehicle (no drug)	6, 7, 7, 8	7	0[c]
artemether (**2**) 6 mg/kg + mefloquine HCl 18 mg/kg	23, 27, 13, 23	21.5	>99.9
mefloquine HCl 18 mg/kg alone	13, 15, 23, 20	17.8	>99.9

[a] Best results are in bold type.

[b] Denotes determination on day 3 after infection.

[c] An average of 10% parasitemia was determined on day 3 after infection.