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The survival times of malaria-infected mice are prolonged more by several new two-carbon-linked artemisinin-derived dimer carbamates than by the trioxane antimalarial drug artemether

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ABSTRACT

Sixteen new artemisinin-derived 2-carbon-linked trioxane dimers were prepared to study chemical structure/antimalarial activity relationships (SAR). Administering a very low single oral dose of only 5 mg/kg of dimer secondary alcohol **6a** or **6b** plus 15 mg/kg of mefloquine hydrochloride prolonged the lives of *Plasmodium berghei*-infected mice to an average of 25 days after infection. This ACT chemotherapy result is of high medicinal significance because the antimalarial efficacy of the popular trioxane drug artemether (**2**) plus mefloquine under the same conditions was significantly lower (only 20 day average survival). NH-aryl carbamate derivatives **7e**, **7i**, and **7j** of 2-carbon-linked dimer alcohol **6b** also significantly outperformed artemether (**2**) in prolonging the survival times (25–27 days) of malaria-infected mice.

Malaria remains a devastating infectious disease, especially in tropical and sub-tropical areas of the world. Attempts at developing vaccines to protect humans from contracting malaria have been only partially successful.[1] Therefore, safe and effective chemotherapy to cure malaria-infected patients is desperately needed. With widespread resistance of *Plasmodium falciparum* malaria parasites to such previously reliable antimalarial drugs like chloroquine,[2–4] antimalarial drugs with new mechanism(s) of action are valuable for chemotherapy of malaria patients.[5,6] In recent years, a dramatic advance has been made with the use of the trioxane artemisinin (**1**) and its derivatives artemether (**2**) and sodium artesunate (**3**, Fig. 1) as fast-acting and highly efficacious antimalarials.[7,8] Combining such fast-acting but short lived trioxanes with known slower-acting but longer-lived nitrogen-containing compounds like mefloquine or lumefantrine is now recommended as standard operating procedure by the World Health Organization (WHO).[9] Such artemisinin combination therapy (ACT) has led to several combinations that are now readily available as over-the-counter drugs. These commercial ACTs, however, typically require multiple dosing for several days in order to achieve full cures.[10–18] Compliance with such a regimen is often problematic. Therefore, a single dose cure is becoming a major goal in modern antimalarial chemotherapy.[19–24] We have recently designed, synthesized, and biologically evaluated a series of new C-10 carbon-linked trioxane dimers some of which completely cure malaria-infected mice using one single-digit oral dose of trioxane along with a non-trioxane drug.[25–29] Artemisinin-derived C-10 carbon-linked dimers having linking units of five,[26] four,[27] and three[28,29] carbon atoms as well as dimers with structurally distinct linking units[30–32] having potent antimalarial activity have been reported. Recently we described preparation of artemisinin-derived 2-carbon-linked dimer ketone **4** (36% overall yield from artemisinin) and of its corresponding curative oxime derivatives **5**.[25] We have now discovered that 2-carbon-linked dimer ketone **4** can be reduced with very high stereocontrol predominantly into secondary alcohol diastereomer **6b**. The new chemical entity alcohol **6b** was easily transformed in one step into a series of fourteen NH-aryl carbamates **7** some of which are more efficacious than the popular monomeric trioxane drug artemether (**2**) in prolonging the survival times of malaria-infected mice (Scheme 1).

It was important to explore the utility of this new 2-carbon-linked dimer scaffold by performing a number of functional group transformations. The dimer ketone **4** was reduced into a pair of diastereomeric alcohols **6a** and **6b** that were easily separable on

Abbreviations: SAR, structure–activity relationship; ACT, artemisinin combination therapy; DIBALH, diisobutylaluminum hydride.

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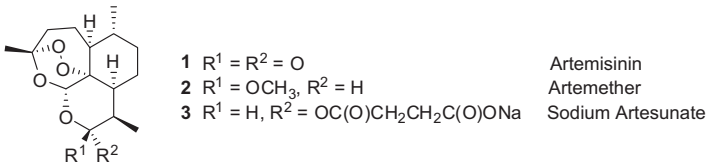


Figure 1. Artemisinin and first generation derivatives.

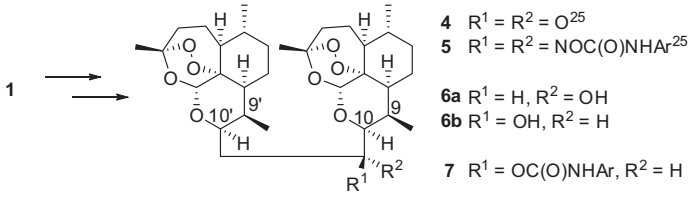
silica gel (Scheme 2). Surprisingly, even with a bulky reducing agent such as diisobutylaluminum hydride (DIBALH), the alcohols **6a** and **6b** were obtained in a 1:1 ratio. However, treatment with commercially available enantiopure (*R*)-CBS-oxazaborolidine[33] **8a** and borane in tetrahydrofuran (BH_3·THF) achieved highly stereoselective reduction of the ketone (98% diastereomeric excess, as determined by ^{1}H NMR of the crude reaction mixture), allowing direct access to diastereomer **6b**. Employing the enantiomeric (*S*)-CBS oxazaborolidine **8b** did not afford the same selectivity, forming both alcohol products **6a** and **6b** in a 62:48 ratio under these conditions.

The more polar alcohol (by analytical TLC) was hypothesized to be the (*S*)-isomer **6b** based on analysis of the proton NMR signals (Fig. 2), on the expected product from the known mechanism of this reaction,[34] and on the structural configuration by molecular model. In the (*S*)-isomer, the alcohol points toward the C5 proton, facilitating a deshielding interaction with this proton (Fig. 2III). Furthermore, the C10 proton would be locked into a pseudo equatorial–axial conformation with the C17 proton (looking down the C10—C17 bond), resulting in a smaller coupling constant (Fig. 2IV). Conversely, the alcohol would be pointed away from the C5 proton in the (*R*)-isomer (Fig. 2I), and the C10–C17 protons would be in a pseudo trans-diaxial orientation (Fig. 2II). Indeed this trend is seen in the ^{1}H NMR. Crystals of **6b** were obtained via slow evaporation of a mixed solvent system (1:4 mixture of dichloromethane and hexanes, with several drops of benzene added). X-ray structure determination confirms the stereochemistry as described in Figure 3. The crystal structure (Fig. 3) also revealed interesting structural characteristics when compared with the ketone precursor.[25] The ketone is a rigid, cage-like structure that locked the endoperoxide pharmacophore on the outside of the molecule, and it was hypothesized that this would be beneficial for antimalarial activity. Reduction to the alcohol seems to contort the six-membered glycosidic ring of the artemisinin core into a twisted conformation. The endoperoxides are also facing in, though flexibility in solution would still be expected.

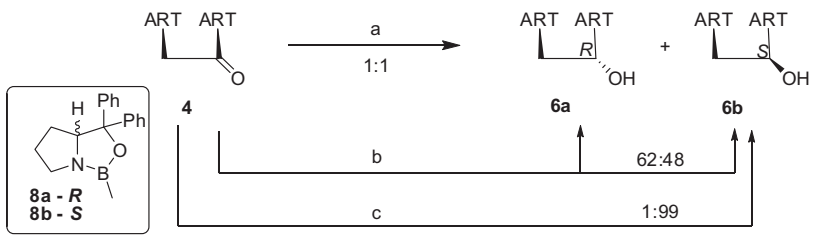
The major diastereomeric alcohol **6b** was converted easily in one step using commercial aryl isocyanates into a series of fourteen NH-aryl carbamates **7** which include phenyl, monofluoro, difluoro, trifluoro, monochloro, monobromo, cyano, and nitro derivatives (Fig. 4). Attempts at preparing NH-alkyl carbamates using commercial alkyl isocyanates were unsuccessful.

Our recent experience showed that all of the new 2-carbon-linked dimer oxime NH-aryl carbamates **5** had significant in vivo antimalarial efficacy.[25] Therefore, we proceeded directly with *in vivo* efficacy evaluation of the new series of OC(O)NH-aryl carbamates **7**. Indeed, as expected, all of these carbamates **7** had significant chemotherapeutic value, with some outperforming the trioxane antimalarial drug artemether (**2**).

To each two-carbon-linked dimer alcohols **6a** and **6b** and carbamates **7** (0.60 mg), 113 μL of 7:3 Tween 80/ethanol with mefloquine hydrochloride (1.80 mg) was added and then diluted with 1087 μL of deionized water for oral administration to 5-week old *C57BL/6J* male mice (from Jackson Laboratory) weighing about 20 g that were infected with *Plasmodium berghei* ANKA strain (2×10^7 parasitized erythrocytes). Each of four mice in a group was treated orally 24 h post-infection with a single dose of 200 μL of diluted compound solution, corresponding to a dose of 5 mg/kg trioxane combined with 15 mg/kg of mefloquine hydrochloride. Determining blood parasitemia levels and monitoring the duration of animal survival compared to survival time of animals receiving no drug are both widely accepted as measures of a drug's antimalarial efficacy. An average of 9.8% parasitemia was observed in the control (infected but no drug treatment) group

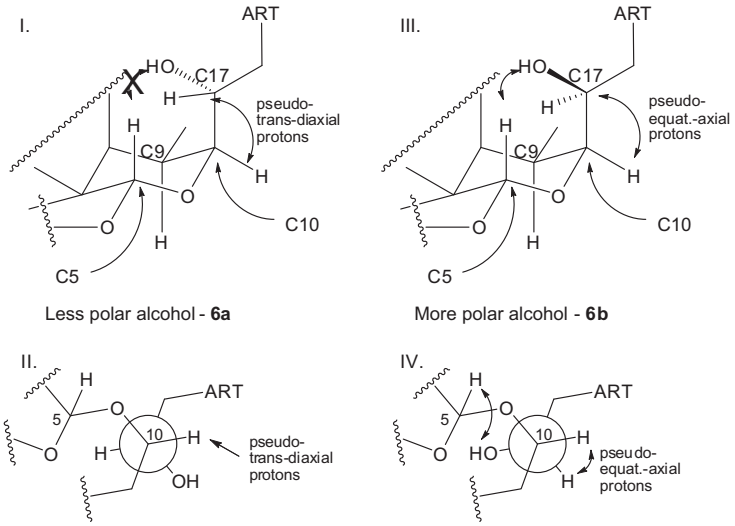


Scheme 1. Two-carbon-linked dimer derivatives.



a) DIBALH, CH_2Cl_2, -78 °C; 98% b) **8b**, BH_3·THF, 0 °C -rt, 99% overall yield for both diastereomers; c) **8a**, BH_3·THF, 0° C - rt, 92%

Scheme 2. Two-carbon-linked dimer alcohols **6**.



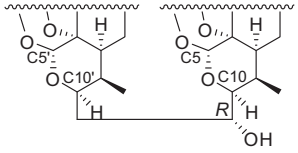
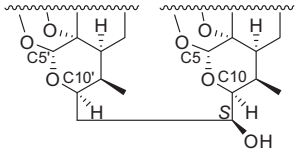
Compound	Structure	C5 δ	C5' δ	C10 δ	$J_{9,10}$	$J_{10,17}$
6a		5.37	5.30	4.73	6.19	10.93
6b		5.54	5.40	4.24	6.73	3.63

Figure 2. Conformational analysis of less polar alcohol **6a** (I and II) and more polar alcohol **6b** (III and IV) using ^{1}H NMR spectroscopy.

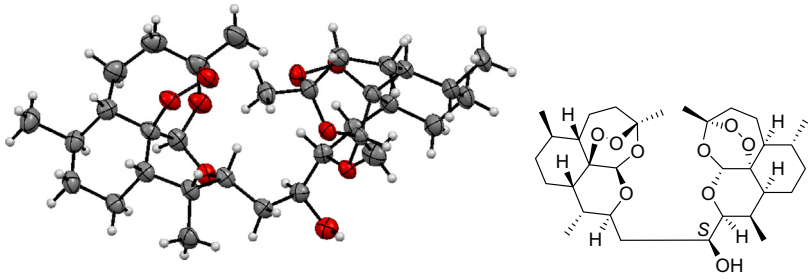
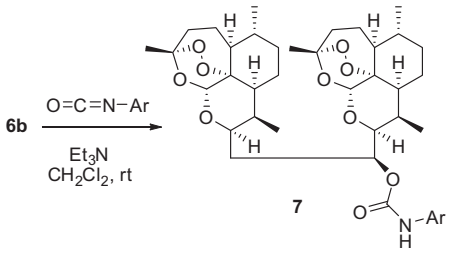


Figure 3. Displacement ellipsoid plot (50% probability level) of two-carbon linked dimer alcohol **6b** given at 110(2) K.

on day 3 post-infection. Infected mice receiving no drug died on an average of 6.5 days post infection. The antimalarial efficacy results of our two-carbon-linked dimer trioxanes as well as controls are summarized in Table 1, which includes the parasitemia levels for mice on day 3 post infection. These data show that all of our two-carbon linked dimer derivatives **6** and **7** acted rapidly to suppress parasitemia. However, not all of the parasites were killed after 3 days which leads to a difference in the efficacy for each individual analog over the full 30 day experiment.

Important conclusions emerge from the data in Table 1. The equipotent two-carbon linked dimer diastereomeric alcohols **6a** and **6b** were more efficacious at prolonging the life of malaria-infected mice (~25 days) compared to artemether (**2**) (~20 days). Monotherapy using only mefloquine hydrochloride (15 mg/kg single oral dose) prolonged the average survival time of malaria-infected mice to about 20 days. Of the fourteen NH-aryl carbamates (**7**) tested, three of these new chemical entities (**7e**, **7i**, and **7j**) were more efficacious at prolonging the life of malaria-infected mice, with average survival time of 25–27 days, compared to artemether (**2**) (~20 day survival). An important characteristic of these three efficacious compounds is the placement of a halogen (Cl or F) in the *meta*-position on the phenyl ring. For example, 3-Cl-4-F-phenyl NH-aryl carbamate **7i** (average survival 25 days) is more efficacious than the corresponding 3-methyl-4-F-phenyl NH-aryl carbamate **7d** (average survival only 13.5 days). Also, 3-Cl-phenyl NH-aryl carbamate **7j** (average survival 27 days) is considerably more efficacious than 4-Cl-phenyl NH-aryl carbamate **7k** (average survival only 18 days). Having two *meta*-fluorines as in carbamate **7f** (average survival 22 days) offers only a small advantage over the corresponding monofluorinated carbamate **7b** (average survival 20 days). The significance of substitution at the 3-position of the phenyl ring has previously been reported with monomeric artemis-



		% yield			% yield
7a	Ar = Ph	60	**7h**	Ar = Ph-4-C F_3	64
7b	Ar = Ph-3-F	78	**7i**	Ar = Ph-3-Cl-4-F	74
7c	Ar = Ph-4-F	65	**7j**	Ar = Ph-3-Cl	65
7d	Ar = Ph-3-Me-4-F	63	**7k**	Ar = Ph-4-Cl	99
7e	Ar = Ph-3,4-F_2	69	**7l**	Ar = Ph-4-Br	60
7f	Ar = Ph-3,5-F_2	71	**7m**	Ar = Ph-4-CN	95
7g	Ar = Ph-3-CF_3	65	**7n**	Ar = Ph-4-NO_2	79

Figure 4. Two-carbon-linked dimer carbamates **7**.

Table 1
In vivo antimalarial efficacy using a single oral dose of trioxane dimer (5 mg/kg) combined with mefloquine hydrochloride (15 mg/kg) in *P. berghei*-infected mice

Trioxane	Survival after infection (days)	Avg survival[a] (days)	% parasitemia suppression[b]
6a	30, 13, 26, 30	**24.8**	>99.9
6b	28, 18, 27, 27	**25**	>99.9
7a	30, 13, 13, 12	17	>99.9
7b	30, 13, 12, 26	20.3	>99.9
7c	30, 13, 16, 16	18.8	>99.9
7d	12, 13, 13, 16	13.5	>99.9
7e	30, 30, 12, 30	**25.5**	>99.9
7f	26, 30, 12, 19	21.8	>99.9
7g	30, 18, 12, 12	18	>99.9
7h	9, 12, 9, 13	10.8	>99.9
7i	30, 26, 30, 13	**24.8**	>99.9
7j	28, 30, 19, 30	**26.8**	>99.9
7k	13, 30, 18, 12	18.3	>99.9
7l	13, 13, 12, 12	12.5	>99.9
7m	16, 12, 30, 13	17.8	>99.9
7n	30, 26, 19, 13	22	>99.9
Controls:			
Infected mice (no drug)	6, 7, 7, 6	6.5	0[c]
Artemether (**2**) plus mefloquine	18, 12, 23, 28	20.3	>99.9
Mefloquine alone	16, 11, 28, 26	20.3	>99.9

[a] Bold entries indicate best results.
[b] Denotes determination on day 3 after infection.
[c] An average of 9.8% parasitemia was determined on day 3 after infection.

inin sulfur derivatives.[35] The 3-Cl-phenyl NH-aryl carbamate **7j** had the highest average survival time of about 27 days. Two of the mice were alive on day 30 with two deaths on days 19 and 28. The 3,4-difluorophenyl NH-aryl carbamate **7e** had an average survival time of about 26 days with 3 mice alive on day 30 and one death on day 12. Compound **7i** (3-Cl-4-F-phenyl NH-aryl carbamate) had an average survival time of about 25 days, with two deaths on days 13 and 26 and two mice alive on day 30. All of the surviving mice in this 30-day study had parasitemia levels between 8–20%.

In conclusion, we have demonstrated here that artemisinin-derived 2-carbon-linked dimer secondary alcohol **6b** is more efficacious as an antimalarial than the popular artemisinin-derived drug artemether. Furthermore, we have established proof of principle showing that 2-carbon-linked dimer secondary alcohol **6b** is a useful platform for generating in one step various NH-aryl carbamates **7**. Three of these new carbamates, acting in vivo perhaps as prodrugs,[36] outperform the trioxane drug artemether in prolonging the survival of malaria-infected mice. We are pursuing other derivatives of dimer secondary alcohol **6b** and will report results in due course.

Acknowledgments

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Supplementary data

Supplementary data (crystallographic information (CCDC 981037), experimental, and tabular spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.059.

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