Malaria-Infected Mice Live Until At Least Day 30 After A New Artemisinin-Derived Thioacetal Thiocarbonate Combined with Mefloquine Are Administered Together In A Single, Low, Oral Dose

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Abstract

In only three steps and in 21–67% overall yields from the natural trioxane artemisinin, a series of 21 new trioxane C-10 thioacetals was prepared. Upon receiving a single oral dose of only 6 mg/kg of the monomeric trioxane **12c** combined with 18 mg/kg of mefloquine hydrochloride, *Plasmodium berghei*-infected mice survived on average 29.8 days after infection. Two of the four mice in this group had no parasites detectable in their blood on day 30 after infection and they behaved normally and appeared healthy. One of the mice had 11% blood parasitemia on day 30, and one mouse in this group died on day 29. Of high medicinal importance, the efficacy of this ACT chemotherapy is much better than (almost double) the efficacy under the same conditions using as a positive control the popular trioxane drug artemether plus mefloquine hydrochloride (average survival time of only 16.5 days).

INTRODUCTION

Malaria kills approximately one million people, mostly children, each year.[1,2,3,4] No vaccine is yet a fully effective prophylactic against malaria.[5,6] Widespread resistance of *Plasmodium falciparum* malaria parasites to chloroquine,[7,8] until recently the most popular malaria chemotherapeutic agent, catapulted rapidly-acting artemisinin (**1**) trioxanes like artemether (**2**) and sodium artesunate (**3**) into use. Typically the trioxane is combined with slower-acting nitrogen-aromatics like mefloquine (**4**), lumefantrine (**5**), and pyronaridine (**6**, Figure 1). Indeed, the World Health Organization (WHO) urges worldwide use of such Artemisinin Combination Therapy (ACT) as the best chemotherapy for malaria patients.[9] Much research has been focused on increasing the efficacy of artemisinin-derived antimalarial drugs through synthetic chemistry.[10,11,12,13]

One of the most promising synthetic (not derived from artemisinin) peroxides is trioxolane OZ439 (**7**) which is now in advanced human clinical trials.[14] Some semi-synthetic artemisinin derivatives also are in clinical trials.[15] Antimalarial drugs now on the market include the following: a fixed combination of **2** and **5**;[16] a fixed combination of **3** and **4**;[17,18] and a fixed combination of **3** and **6**.[19,20] Each of these three combinations requires patients to take pills daily for several days.[16,17,18,19,20,21] Adherence to such a schedule of ACT multiple dosing often is not followed faithfully, leading to ineffective chemotherapy and to increased likelihood of resistance developing.[22,23,24] To overcome such non-adherence to a repeated dosing regimen, curing malaria patients with a single oral dose of drug is a very important goal.[25,26,27,28,29] Toward that goal, we have reported that malaria-infected mice are cured by a single, oral, 6 mg/kg dose of several new artemisinin derivatives combined with 18 mg/kg of mefloquine hydrochloride.[30,31]

Some thioacetal glycosides[32] and thiosugar hemithioacetals[33] have potent biological activities, and a thioacetal has been developed as thiol protecting group.[34] Here we disclose a new series of artemisinin-derived monomeric C-10 thioacetal carbonates, thiocarbonates, nitrogen-heterocycles, and a dimeric trioxane C-10 thioacetal amide. A trioxane monomer carbonate[35] as well as a dimer carbonate have been reported,[36] as well as some C-10 thioacetal derivatives of artemisinin.[37,38,39,40,41]

RESULTS AND DISCUSSION

Chemistry

Thioacetals are known to be more hydrolytically stable than the corresponding acetals.[42,43] Since some trioxane acetals are efficacious antimalarial drugs, like **2** and **3**, trioxane thioacetals were expected to be of potential value as new antimalarials. Thus, our interest was to synthesize easily and to test some trioxane thioacetals for antimalarial activity in *Plasmodium berghei*-infected mice.

We prepared a series of C-10α thioacetals in the yields shown in Scheme 1 in three facile steps from **1**. The first step is reduction of **1** into dihydroartemisinin (**8**)[44] without destroying the crucial trioxane peroxide pharmacophore.[45] Introduction of the Lewis acid boron trifluoride diethyl etherate to **8** generates an intermediate oxocarbenium ion. A variety of nucleophiles are known to react with oxocarbenium ions and, as predicted, the reaction with thiols proceeds smoothly to produce the desired C-10 thioacetals. The thiols used are commercially available bifunctional mercaptoalcohols and a mercaptocarboxylic acid, which leaves a synthetic handle, the hydroxyl or carboxylic acid group, for further manipulation and SAR studies.

The stereochemistry at the C-10 position in thioacetals **9** and **10** was determined to establish the diastereoselectivity of the thioacetalization reaction. Since the reactive C-10 site of the intermediate oxocarbenium ion is planar, the nucleophile could attack from either face of the molecule. Consequently, each synthesized thioacetal could be a mixture of both C-10α and C-10β diastereomers. Determination of the stereochemistry of the major diastereomer formed was achieved by comparing ^{1}H NMR coupling constants between the C-10 proton and the adjacent C-9 proton in the crude thioacetals (4–9:1 ratio of diastereomers). After column chromatography purification, the major diastereomers **9a**, **9b**, and **10** were isolated in the yields shown. In each case the major diastereomer formed was the C-10α thioacetal based on its C-10 to C-9 coupling constant of 9–11 Hz. These values were characteristic of C-10α thioacetals in previous reports.[37,38,39,40,41] Several C-10α and C-10β phenylthioacetals have been shown to be antimalarially efficacious in mice, with the C-10α thioacetals being more potent than the C-10β thioacetals.[37,38,39,40,41]

Carbonates are more stable than esters,[46,47] and the carbonate functional group has been used effectively before as a prodrug, subsequent hydrolysis of which releases the parent alcohol.[46,47] The steps in Scheme 2 are high-yielding and provide access to a small library of thioacetal carbonates. Thioacetal carbonates **11** were synthesized from the parent alcohol **9** by reaction with base and a chloroformate (Scheme 2). Besides aliphatic and aromatic carbonates **11a–11h**, sulfone carbonate **11i** was of interest because of a report demonstrating the usefulness of this particular functionality in a prodrug.[48]

With the intention to test some compounds that were similar in structure to the thioacetal carbonates, we synthesized the series C-10α thioacetal thiocarbonates **12a–c** as well as C-10α thioacetal xanthate ester **12d** from diastereomerically pure thioacetal alcohol **9a**. Preparation of **12a–c** involved similar chemistry to that for the thioacetal carbonates, although change of solvent as well as base was necessary to optimize reaction conditions. In the case of **12d**, **9a** was treated with base, carbon disulfide, and methyl iodide in tetrahydrofuran (Scheme 3).

Since the development of chloroquine in the 1940s, nitrogen-containing heterocycles have been a cornerstone of antimalarial drug research.[49,50,51] These moieties possess unique acid/base properties and mimic many important biological molecules. With these characteristics in mind, we prepared nitrogen-heterocycle-containing C-10 thioacetal ethers **13** (Scheme 4) and benzimidazole-containing C-10 thioacetal amides **14** (Scheme 5).

Making dimers of drug molecules is a common practice in medicinal chemistry; for every one molecule of drug, two pharmacophore moieties reach the active site within the body.[52,53] Therefore, dimer drug compounds are sometimes considerably more efficacious than their monomer counterparts.[52,53] Due to recent success with amide-containing monomeric trioxanes as antimalarials,[30,54] we synthesized bis-amide bisthioacetal dimer **15** (Scheme 6).

Based on our previous *in vivo* results, we have observed some trends between log P values and antimalarial efficacy.[30,31] Typically, monomeric trioxanes are most efficacious when the log P value is between 4 and 6. Dimeric trioxanes have displayed optimal results when the log P value is between 7 and 9.

Although literature suggested that our new C-10 thioacetals **9**–**15** would be much more stable to acid than the analogous acetals,[42,43] we subjected lead trioxane **12c** to a series of stability tests since it was the most efficacious of our antimalarial thioacetals. First, hydrolytic stability was tested based on a published protocol that simulated stomach acidic conditions, which is important for a drug that is administered orally.[55] Trioxane **12c** was dissolved a solution of aqueous hydrochloric acid and acetonitrile (pH = 2) and heated in a temperature-controlled oil bath set to 37 °C. This solution was allowed to stir for 24 h and was periodically analyzed by TLC. Additionally, the solution was analyzed by ^{1}H NMR after completion of the 24 h. Since we knew that the product formed through hydrolysis of the C-10 thioacetal functionality would be **8**, we compared the ^{1}H NMR spectrum of **12c** after 24 h to that of **8** and to that of parent alcohol **9a**. Less than 2% hydrolysis of **12c** had occurred. Similarly, **9a** and **9b** were stable to hydrolysis under the same conditions. Full experimental details of the hydrolysis study are included in the supporting information.

Because ACT is used predominantly in tropical areas where malaria is endemic, it was important to determine the thermal stability of lead trioxane **12c** at 60 °C, neat, for 7 days; TLC as well as ^{1}H NMR showed no decomposition.

Biology

To each thioacetal trioxane (0.64 mg), 100 μL of 7:3 Tween 80:ethanol with mefloquine hydrochloride (1.92 mg) was added. This mixture was then diluted with 965 μof water for oral administration to 5-week old C57BL/6J male mice (from the Jackson Laboratory) weighing about 20 g that were infected with *P. 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 6 mg/kg trioxane combined with 18 mg/kg of mefloquine hydrochloride. Determining blood parasitemia levels as well as 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 8.8% blood parasitemia was observed in the control (no drug) group on day 3 post infection. Infected animals receiving no drug died on an average of 8 days post infection. The antimalarial efficacy results of our C-10 thioacetals as well as controls are summarized in Table 4, which includes the parasitemia levels for mice on day 3 post infection.

Based on these data and as expected for artemisinin-derived trioxanes,[10,11,12,13] all of our new C-10α thioacetals acted rapidly to suppress parasitemia, with almost complete suppression of parasitemia as determined on day 3 post infection. However, not all of the parasites were killed after 3 days which leads to a difference, sometimes substantial, in efficacy for each individual analog over the full 30 day experiment.

Also based on these data, it is clear that each of the four C-10 thioacetals **11h** (25.3 days), **12a** (24.5 days), **12c** (29.8 days), and **14a** (26.3 days) in combination with mefloquine hydrochloride prolonged the mouse average survival time by more than one week (>23.5 days) compared to the survival time of the artemether plus mefloquine hydrochloride control (16.5 days). Mefloquine hydrochloride alone at 18 mg/kg gave an average survival time of only 14 days.

Most impressively, trioxane **12c** produced a partial cure with an average survival time of 29.8 days. Two of the four mice in this group showed no signs of parasitemia in their blood on day 30 post infection and behaved normally. One mouse in this group had 11% parasitemia on day 30, and one mouse died on day 29.

CONCLUSIONS

In conclusion, several artemisinin-derived C-10α thioacetals were found to be more efficacious as antimalarials than artemether. Four of the new thioacetals (**11h**, **12a**, **12c**, and **14a**) combined with mefloquine hydrochloride prolonged the life of *P. berghei*-infected mice by at least one week longer than the artemether plus mefloquine hydrochloride control. Remarkably, when administered only once as a single, oral dose of 6 mg/kg plus 18 mg/kg of mefloquine hydrochloride, trioxane **12c** was highly efficacious with an average survival time of 29.8 days, almost double the average survival time (16.5 days) achieved by the popular antimalarial drug artemether plus mefloquine positive control using the same protocol.

EXPERIMENTAL SECTION

The purity of compounds **11h**, **12a**, **12c**, and **14a** was determined to be >98% by HPLC. HPLC data were acquired using a Varian ProStar 210 two-pump system with a Sedex Model 75 Evaporative Light Scattering Detector (ELSD). A Varian 250×4.6mm $\times$ ¼" Microsorb-MV 100-5 Si column was used. All other instrumentation details are included in the Supporting Information.

Thioacetal Alcohol 9a

An oven-dried, 5 dram vial, equipped with a magnetic stir bar, under argon was charged with **8**[44] (250 mg, 0.88 mmol, 1.0 equiv) and anhydrous dichloromethane (10 mL). 3-Mercaptopropanol (89 mg, 0.97 mmol, 1.1 equiv) was added and allowed to stir for 10 min at 50 °C, under argon. Boron trifluoride diethyl etherate (0.125 mL, 0.88 mmol, 1.0 equiv) was added dropwise and the reaction was allowed to stir under argon at 50 °C for 30 min. After 30 min, the reaction was quenched with water (5 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated on a rotary evaporator at room temperature. The ^1H NMR of the crude reaction mixture indicated a mixture of 10-α and 10-β diastereomers in a ratio of 9:1 (α:β). The crude amorphous solid was purified via column chromatography (5–10% ethyl acetate in hexanes) to afford **9a** as a white solid (268 mg, 85% yield). Mp = 108.6–110.0 °C; $[\alpha]_D^{23.3}$ +31.49 (c. 0.58, $CHCl_3$); IR (thin film) ν 3445, 2926, 2871, 2363, 1716, 1586, 1446, 1378, 1279, 1195, 1126, 1035, 928, 900, 878 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.28 (s, 1H), 4.52 (d, J = 10.7 Hz, 1H), 3.87 – 3.76 (m, 2H), 2.94 (ddd, J = 13.5, 7.7, 5.8 Hz, 1H), 2.80 (s, 1H), 2.70 (ddd, J = 13.3, 7.1, 5.9 Hz, 1H), 2.59 (ddd, J = 11.1, 7.3, 4.3 Hz, 1H), 2.33 (ddd, J = 14.6, 13.4, 4.0 Hz, 1H), 1.98 (ddd, J = 14.6, 4.8, 2.8 Hz, 1H), 1.92 – 1.63 (m, 4H), 1.57 (dt, J = 13.5, 4.3 Hz, 1H), 1.50 – 1.15 (m, 7H), 1.10 – 0.95 (m, 1H), 0.91 (dd, J = 12.2, 6.7 Hz, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.7, 92.6, 80.8, 80.7, 59.8, 51.9, 46.1, 37.6, 36.4, 34.2, 32.2, 31.9, 25.9, 24.9, 24.1, 21.5, 20.4, 15.2; HRMS (FAB) *m/z* calcd for $C_{18}H_{30}O_5S$ $[M + H]^+$ 359.1892, found 359.1888.

Thioacetal Alcohol 9b

A 2-dram vial, equipped with magnetic stir bar and argon inlet adaptor, was charged with **8** (0.18 mmol, 50 mg) in anhydrous dichloromethane (2 mL). 6-Mercaptohexanol (0.19 mmol, 26 mg) was added in one portion neat directly to the stirring solution. Boron trifluoride diethyl etherate (0.18 mmol, 25 mg) was added dropwise via a needle and plastic syringe and the reaction was stirred for 20 min. The reaction was quenched with water (2 mL) and extracted with dichloromethane (3 × 2 mL). The organic layers were pooled, dried with magnesium sulfate, vacuum filtered and concentrated via rotary evaporation at room temperature. The crude residue was purified by flash column chromatography on silica, eluting with a gradient mobile phase (5–10% ethyl acetate in hexane) to yield **9b** as a clear amorphous solid (45 mg, 64%). $[\alpha]_D^{24.0}$ +14.6 (c. 0.65, $CHCl_3$); IR (thin film) ν 3458, 2927, 2871, 1455, 1377, 1128, 1037, 928, 879, 829, 666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.28 (s, 1H), 4.51 (d, J = 10.7 Hz, 1H), 3.63 (br t, J = 6.6 Hz, 2 H), 2.78 (ddd, J = 12.5, 8.3, 6.3 Hz, 1H), 2.72 – 2.51 (m, 2H), 2.36 (ddd, J = 14.6, 13.3, 4.0 Hz, 1H), 2.01 (ddd, J = 14.5, 4.9, 2.9 Hz, 1H), 1.90 – 1.83 (m, 1H), 1.77 – 1.15 (m, including a singlet at 1.41, 19 H), 1.07 – 0.99 (m, 1H), 0.95 (d, J = 6.0 Hz, 3H), 0.92 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.4, 92.4, 80.7, 80.6, 63.0, 52.0, 46.2, 37.5, 36.4, 34.2, 32.7, 31.9, 29.9, 28.8, 28.3, 26.1, 25.4, 24.9, 21.4, 20.4, 15.2; HRMS (FAB) *m/z* calcd for $C_{21}H_{36}O_5S$ $(M+H)+$ 401.2362, found 401.2355.

Thioacetal Carboxylic Acid 10

An oven-dried, 2 dram vial, equipped with a magnetic stir bar, under argon was charged with **8** (100 mg, 0.35 mmol, 1.0 equiv) and anhydrous dichloromethane (4 mL). 3-Mercaptopropionic acid (40 mg, 0.39 mmol, 1.1 equiv) was added and allowed to stir for 10 min at 50 °C, under argon. Boron trifluoride diethyl etherate (49.6 μL, 0.35 mmol, 1.0 equiv) was added dropwise and the reaction was allowed to stir under argon at 0 °C for 30 min. After 30 min, the reaction was quenched with water (5 mL), extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator at room temperature. The ^1H NMR of the

crude reaction mixture indicated a mixture of 10-α and 10-β diastereomers in a ratio of 6:1 (α:β). The crude amorphous solid was purified via column chromatography (5–10% ethyl acetate in hexanes) to afford **10** as an amorphous solid (96 mg, 73% yield). $[\alpha]_D^{22.6}$ +26.08 (c 1.1, CHCl$_3$); IR (thin film) ν 2926, 2872, 1707, 1449, 1378, 1268, 1230, 1195, 1128, 1086, 1069, 1036, 959, 928, 900, 879 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.29 (s, 1H), 4.57 (d, J = 10.8 Hz, 1H), 3.16 – 2.94 (m, 1H), 2.95 – 2.75 (m, 3H), 2.73 – 2.55 (m, 1H), 2.36 (ddd, J = 14.5, 13.2, 4.0 Hz, 1H), 2.01 (ddd, J = 14.5, 4.9, 2.9 Hz, 1H), 1.95 – 1.80 (m, 1H), 1.80 – 1.54 (m, 3H), 1.54 – 1.15 (m, 7H), 1.13 – 0.98 (m, 1H), 0.94 (dd, J = 9.2, 6.6 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 176.8, 104.7, 92.3, 81.1, 80.6, 51.9, 46.1, 37.5, 36.4, 35.7, 34.2, 31.4, 25.9, 24.9, 23.6, 21.4, 20.4, 15.1; HRMS (FAB) *m/z* calcd for C$_{18}$H$_{28}$O$_6$S [M + H]$^+$ 373.1685, found 373.1669.

Thioacetal Carbonate 11h

An oven-dried 2-dram vial, equipped with magnetic stir bar and argon gas inlet needle, was charged with **9b** (17.7 mg, 0.044 mmol, 1 equiv) in dry CH$_3$CN (1 mL). Sodium hydride (2 mg, 0.088 mmol, 2 equiv) was added as a solid in one portion and the reaction was stirred at room temperature for 30 min. Propargyl chloroformate (21 mg, 0.176 mmol, 4 equiv) was added dropwise via syringe. The reaction was stirred for 24 h at room temperature, and then more sodium hydride (2 mg, 0.088 mmol, 2 equiv) and propargyl chloroformate (21 mg, 0.176 mmol, 2 equiv) were added. After 12 h, the reaction was quenched with water (2 mL) and extracted with CH$_2$Cl$_2$ (3 × 2 mL). The organic layers were pooled, dried with MgSO$_4$ (*ca.* 1 g), vacuum filtered, and concentrated via rotary evaporation at room temperature. The crude residue was purified by flash chromatography on silica gel to yield **11h** as a clear oil (13.4 mg, 64%). $[\alpha]_D^{22.7}$ +11.8 (c. 0.59, CHCl$_3$); IR (thin film) ν 3279, 2973 – 2851, 1751, 1377, 1279, 1259, 1229, 1128, 1051, 1037, 1017, 928, 880, 678 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.27 (s, 1H), 4.71 (d, J = 2.4 Hz, 2H), 4.51 (d, J = 10.7 Hz, 1H), 4.16 (t, J = 6.6 Hz, 2H), 2.77 (ddd, J = 12.5, 8.2, 6.3 Hz, 1H), 2.68 – 2.59 (m, 2H), 2.52 (t, J = 2.4 Hz, 1H), 2.36 (ddd, J = 14.6, 13.3, 4.0 Hz, 1H), 2.00 (ddd, J = 14.4, 4.9, 2.9 Hz, 1H), 1.92 – 1.81 (m, 1H), 1.75 – 1.52 (m, 8H), 1.50 – 1.19 (m, 10H), 1.10 – 0.98 (m, 1H), 0.96 – 0.90 (m, 7H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 154.8, 104.4, 92.4, 80.7, 80.6, 75.7, 75.7, 68.8, 55.2, 52.0, 46.2, 37.5, 36.4, 34.2, 31.9, 29.9, 28.7, 28.6, 28.3, 26.1, 25.4, 24.9, 21.5, 20.4, 15.2; HRMS (FAB) *m/z* calcd for C$_{25}$H$_{39}$O$_7$S (M+H)+ 483.2417, found 483.2408.

Thioacetal Thiocarbonate 12a

An oven-dried, 2 dram vial, equipped with a magnetic stir bar, under argon was charged with **9a** (10 mg, 0.028 mmol, 1.0 equiv) and dichloromethane (1.0 mL). Pyridine (3 mg, 0.42 mmol, 1.5 equiv) was added and the mixture was allowed to stir for 15 min. After 15 min *S*-methyl chlorothioformate (5 mg, 0.042 mmol, 1.5 equiv) was added and the reaction was allowed to stir under argon for 24 hr. After 24 hr the reaction was quenched with water (5 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried over MgSO$_4$, and concentrated on a rotary evaporator at room temperature. The crude amorphous solid was purified via column chromatography (10% ethyl acetate in hexanes) to afford **12a** as a white amorphous solid (9.8 mg, 81% yield). $[\alpha]_D^{22.1}$ –2.40 (c. 0.55, CHCl$_3$); IR (thin film) ν 2925, 2871, 1709, 1452, 1377, 1149, 1033, 926, 877, 826 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.27 (s, 1H), 4.52 (d, J = 10.8 Hz, 1H), 4.35 (td, J = 6.3, 1.0 Hz, 2H), 2.87 (dt, J = 13.6, 6.9 Hz, 1H), 2.76 – 2.53 (m, 2H), 2.33 (s, 4H), 2.14 – 1.96 (m, 3H), 1.87 (ddt, J = 13.5, 6.7, 3.3 Hz, 1H), 1.77 – 1.65 (m, 2H), 1.64 – 1.21 (m, 8H), 1.11 – 0.98 (m, 1H), 0.93 (dd, J = 9.8, 6.6 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 171.7, 104.4, 92.4, 80.8, 80.5, 66.4, 51.9, 46.2, 37.5, 36.4, 34.2, 31.8, 29.3, 26.1, 24.9, 21.4, 20.4, 15.2, 13.6; HRMS (FAB) *m/z* calcd for C$_{20}$H$_{32}$O$_6$S$_2$ [M + Na]$^+$ 455.1538, found 455.1532.

Thioacetal Thiocarbonate 12c

An oven-dried, 2 dram vial, equipped with a magnetic stir bar, under argon was charged with **9a** (10 mg, 0.028 mmol, 1.0 equiv) and dichloromethane (1.0 mL). Pyridine (3 mg, 0.042 mmol, 1.5 equiv) was added and the mixture was allowed to stir for 15 min. After 15 min *S*-*t*-butyl chlorothioformate (6 mg, 0.042 mmol, 1.5 equiv) was added and the reaction was allowed to stir under argon for 24 hr. After 24 hr the reaction was quenched with water (5 mL) and extracted with dichloromethane (3×5 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated on a rotary evaporator at room temperature. The crude amorphous solid was purified via column chromatography (10% ethyl acetate in hexanes) to afford **12c** as a white solid (10.9 mg, 82% yield). Mp = 102.4–103.9 °C; $[\alpha]_D^{22.1}$ +2.40 (c. 0.29, $CHCl_3$); IR (thin film) ν 2961, 2922, 2872, 1706, 1455, 1377, 1125, 1036, 927, 879, 828 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.27 (s, 1H), 4.52 (d, J = 10.8 Hz, 1H), 4.30 (td, J = 6.4, 0.9 Hz, 2H), 2.92 – 2.78 (m, 1H), 2.76 – 2.52 (m, 2H), 2.43 – 2.28 (m, 1H), 2.11 – 1.95 (m, 3H), 1.94 – 1.81 (m, 1H), 1.79 – 1.66 (m, 2H), 1.64 – 1.37 (m, 15H), 1.37 – 1.20 (m, 2H), 1.12 – 0.98 (m, 1H), 0.93 (dd, J = 9.9, 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 104.4, 92.4, 80.8, 80.5, 65.3, 51.9, 47.2, 46.2, 37.5, 36.4, 34.2, 31.8, 30.3, 29.3, 26.1, 25.0, 24.9, 21.4, 20.4, 15.2; HRMS (FAB) *m/z* calcd for $C_{23}H_{38}O_6S_2$ [M + H]$^+$ 475.2188, found 475.2174.

Thioacetal Benzimidazole Amide 14a

To a 2.5 mL microwave vial was added **10** (15 mg, 0.040 mmol), EDC (8.5 mg, 0.044 mmol), DMAP (5.4 mg, 0.044 mmol), and commercially available 2-aminobenzimidazole (5.86 mg, 0.044 mmol) and dissolved in CH_2Cl_2 (1 mL) under an Ar blanket. The solution was heated to 60 °C for 1.5 hours via microwave irradiation, at which point it was quenched with saturated $NaHCO_3$ (2 mL) and extracted with CH_2Cl_2 (3×1.5 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The crude oil was purified by column chromatography (0–4% MeOH/CH_2Cl_2) to provide **14a** as a colorless, amorphous solid (88% yield, 17.3 mg, 0.035 mmol). $[\alpha]_D^{23.9}$ = –69.6 (c = 1.550, $CHCl_3$); FTIR (thin film) ν 3341, 2926, 2872, 1686, 1633, 1578, 1456, 1271, 1127, 1085; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.27 (s, 1H), 7.52 (s, 3H), 7.25 – 7.16 (m, 2H), 5.19 (s, 1H), 4.51 (d, J = 10.8 Hz, 1H), 3.33 – 3.15 (m, 2H), 3.10 – 2.87 (m, 2H), 2.62 (dqd, J = 11.0, 7.2, 4.0 Hz, 1H), 2.33 (td, J = 13.3, 3.9 Hz, 1H), 2.04 – 1.91 (m, 1H), 1.90 – 1.76 (m, 1H), 1.68 – 1.49 (m, 3H), 1.48 – 1.36 (m, 1H), 1.35 (s, 3H), 1.29 – 1.13 (m, 3H), 1.11 – 0.95 (m, 1H), 0.95 – 0.92 (d, J = 6.0 Hz, 3H), 0.86 (d, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 172.4, 147.6, 122.2, 104.5, 92.2, 92.1, 89.6, 80.4, 80.3, 51.6, 45.8, 37.39, 37.3, 36.22, 34.0, 31.1, 25.8, 24.6, 24.0, 21.0, 20.12, 18.4, 14.8, 6.8.; HRMS (ESI) calc'd for $C_{25}H_{34}N_3O_5S$ (M+H)$^+$ 488.2219, found 488.2217

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS USED

ACT   artemisinin combination therapy

WHO   world health organization

DHA	dihydroartemisinin
EDC	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
DMAP	4-dimethylaminopyridine

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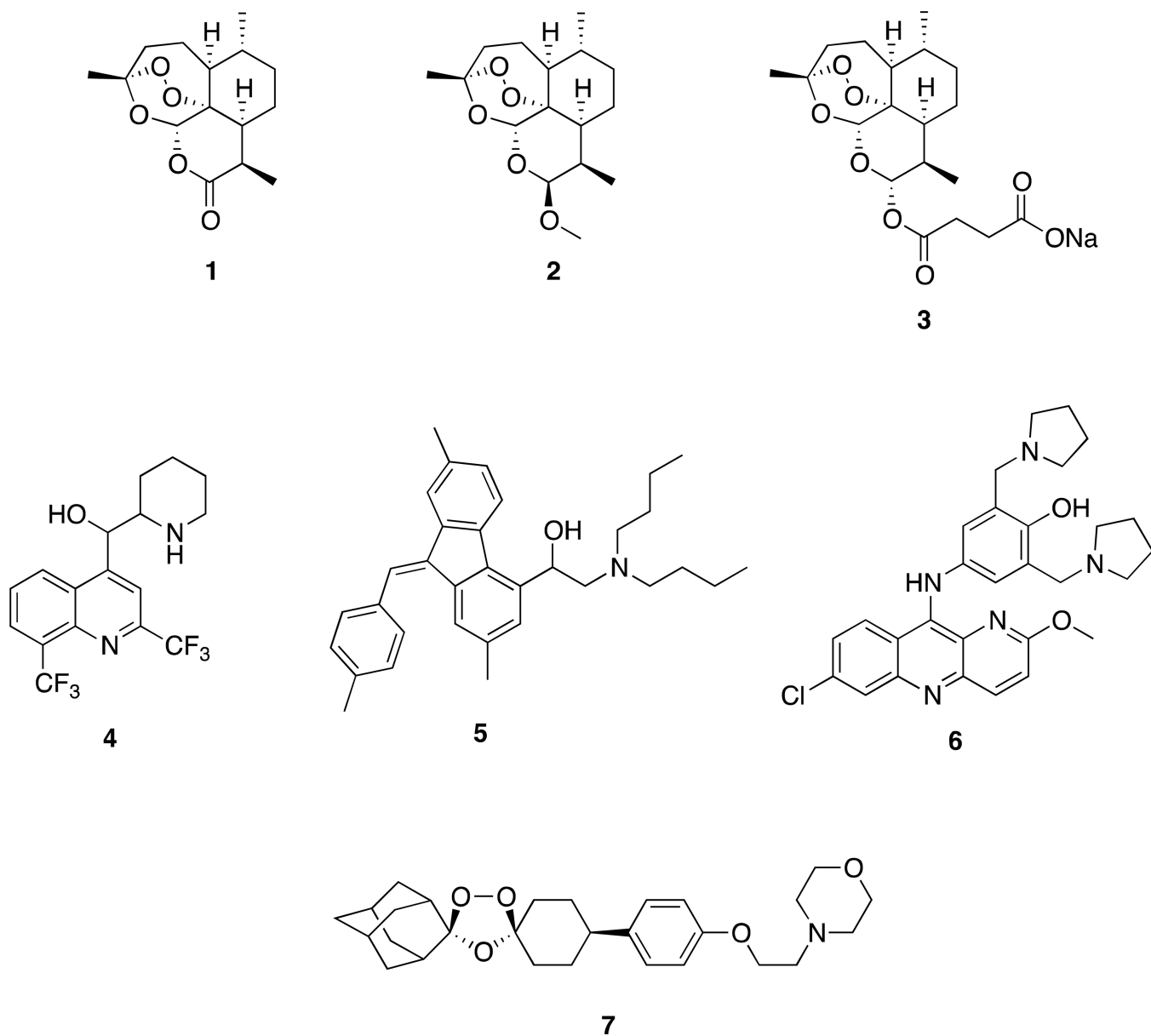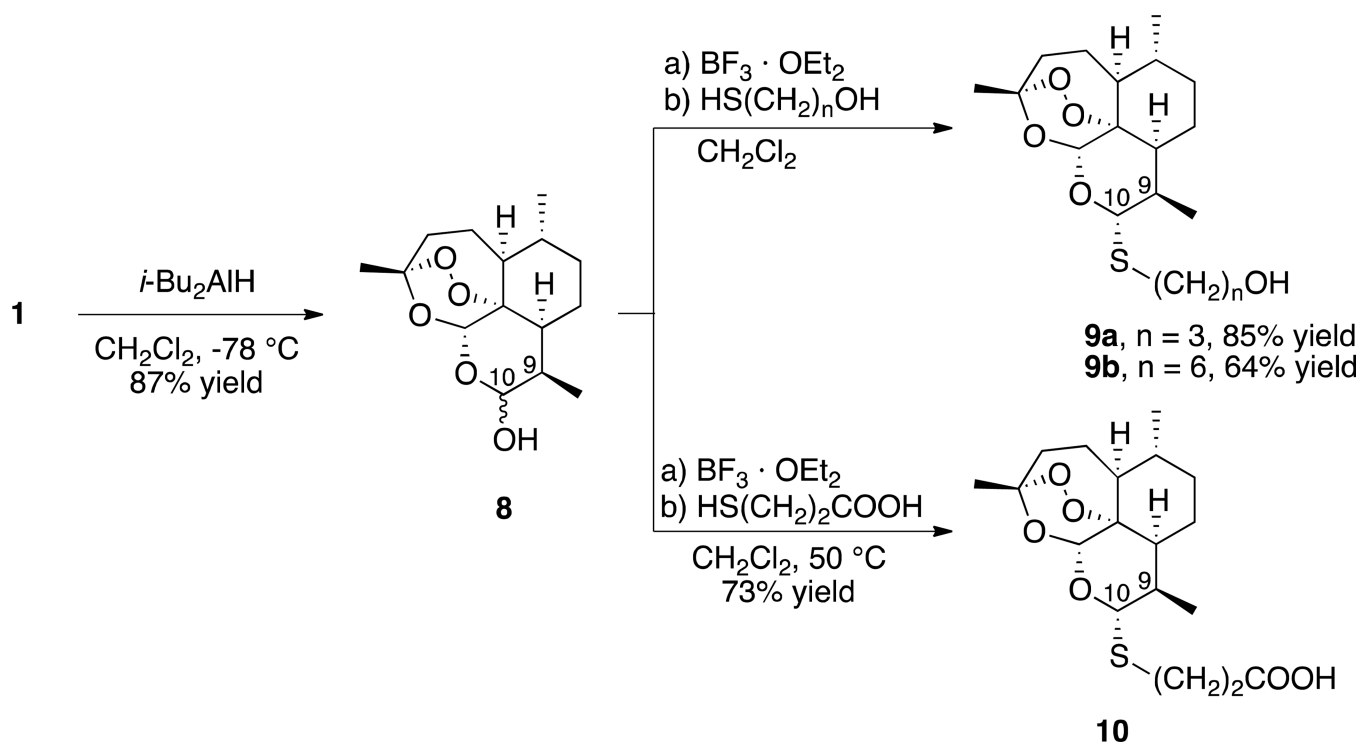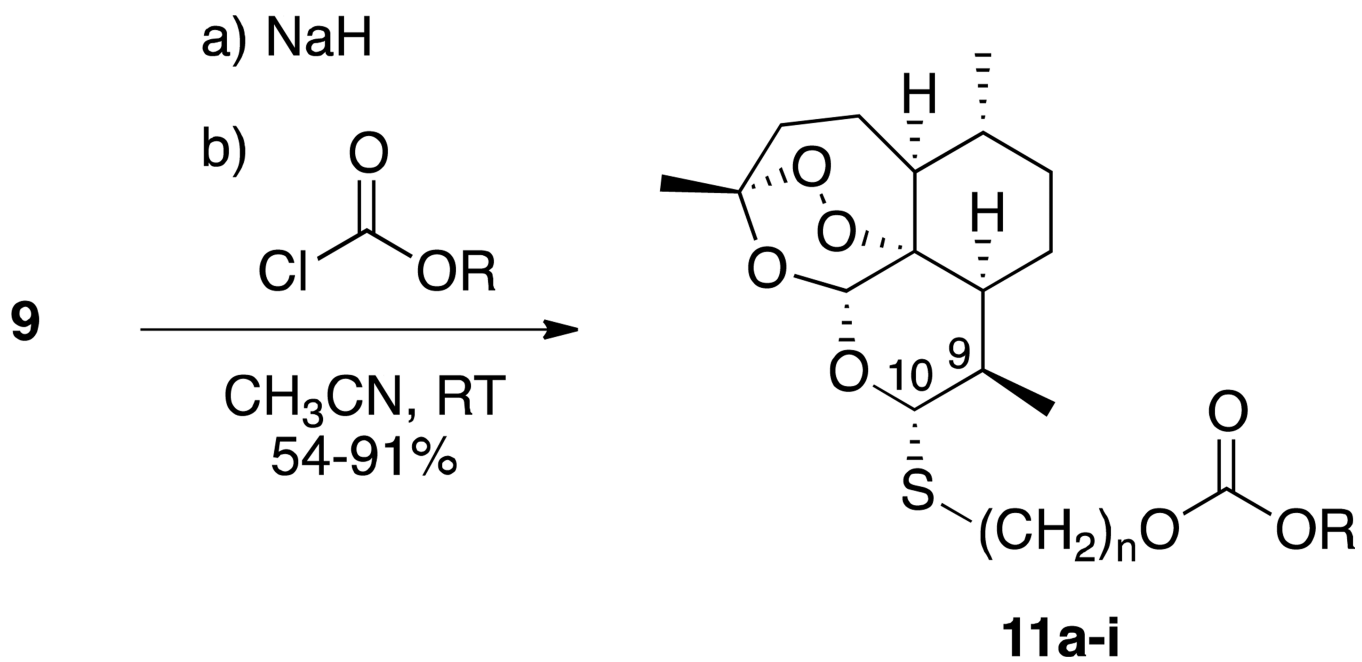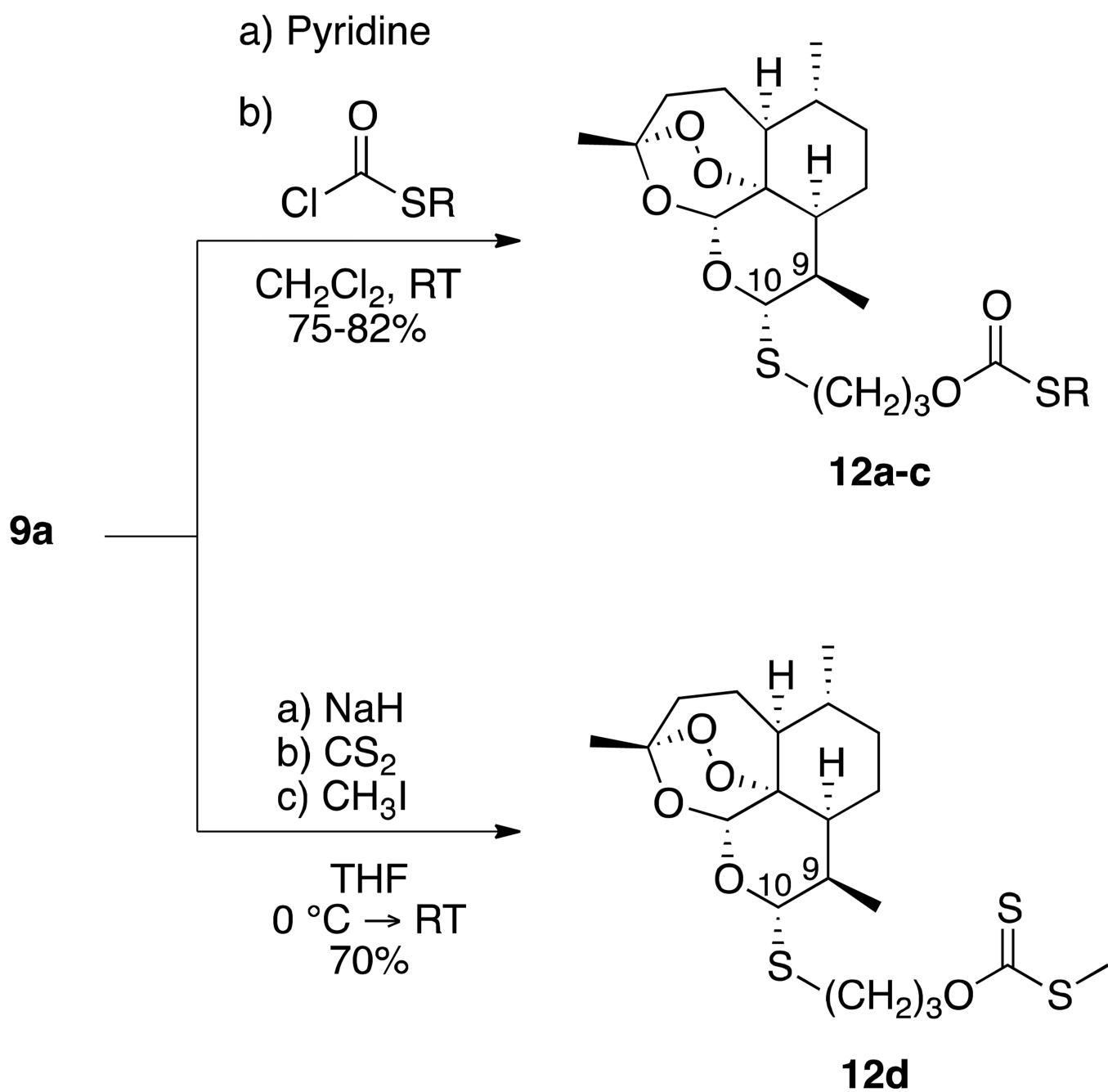
1

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Figure 1.

Scheme 1.

a) NaH

b)

9

CH$_3$CN, RT
54-91%

11a-i

Scheme 2.

a) Pyridine

b)

CH$_2$Cl$_2$, RT
75-82%

9a

12a-c

a) NaH
b) CS$_2$
c) CH$_3$I

THF
0 °C → RT
70%

12d
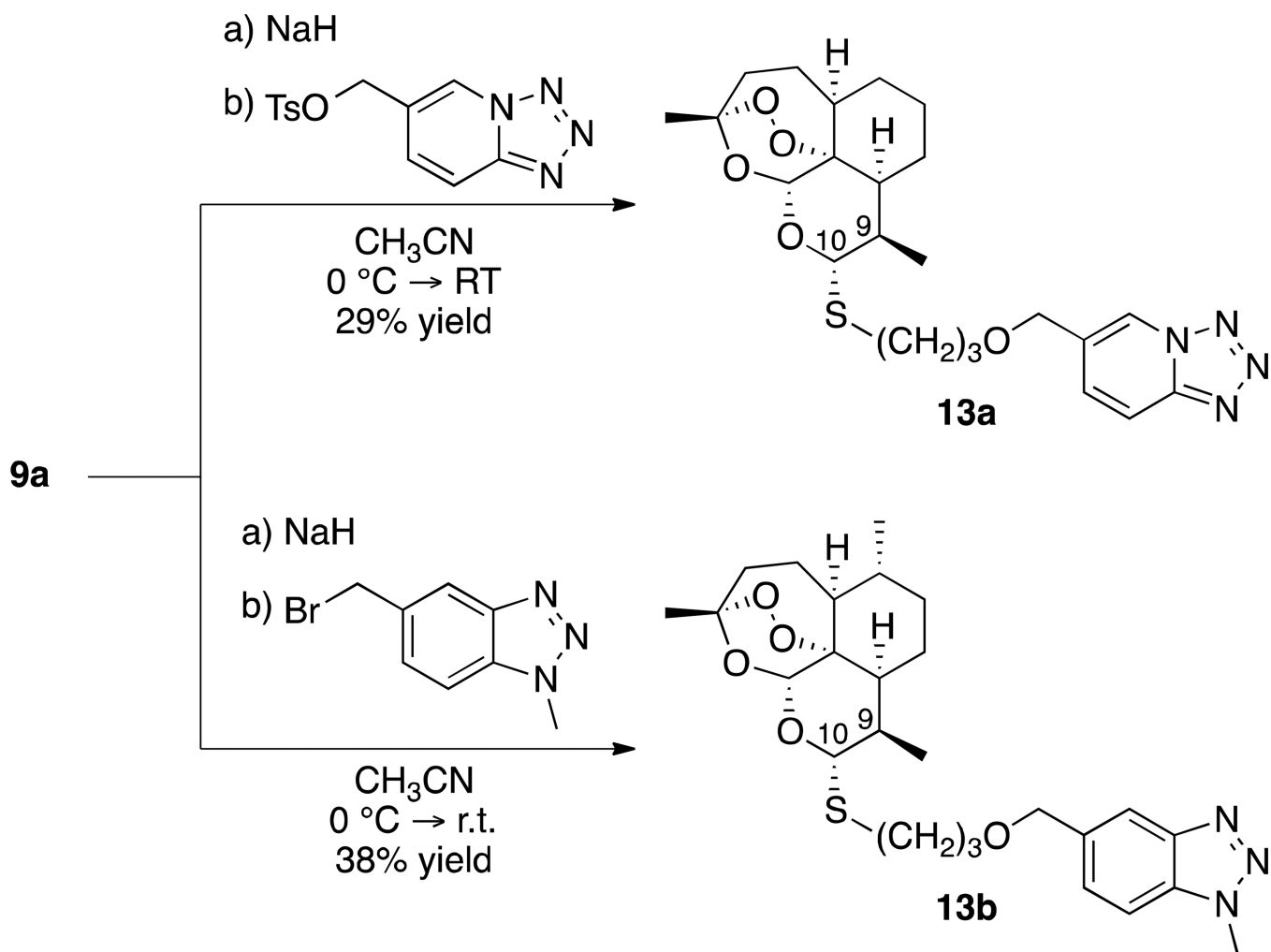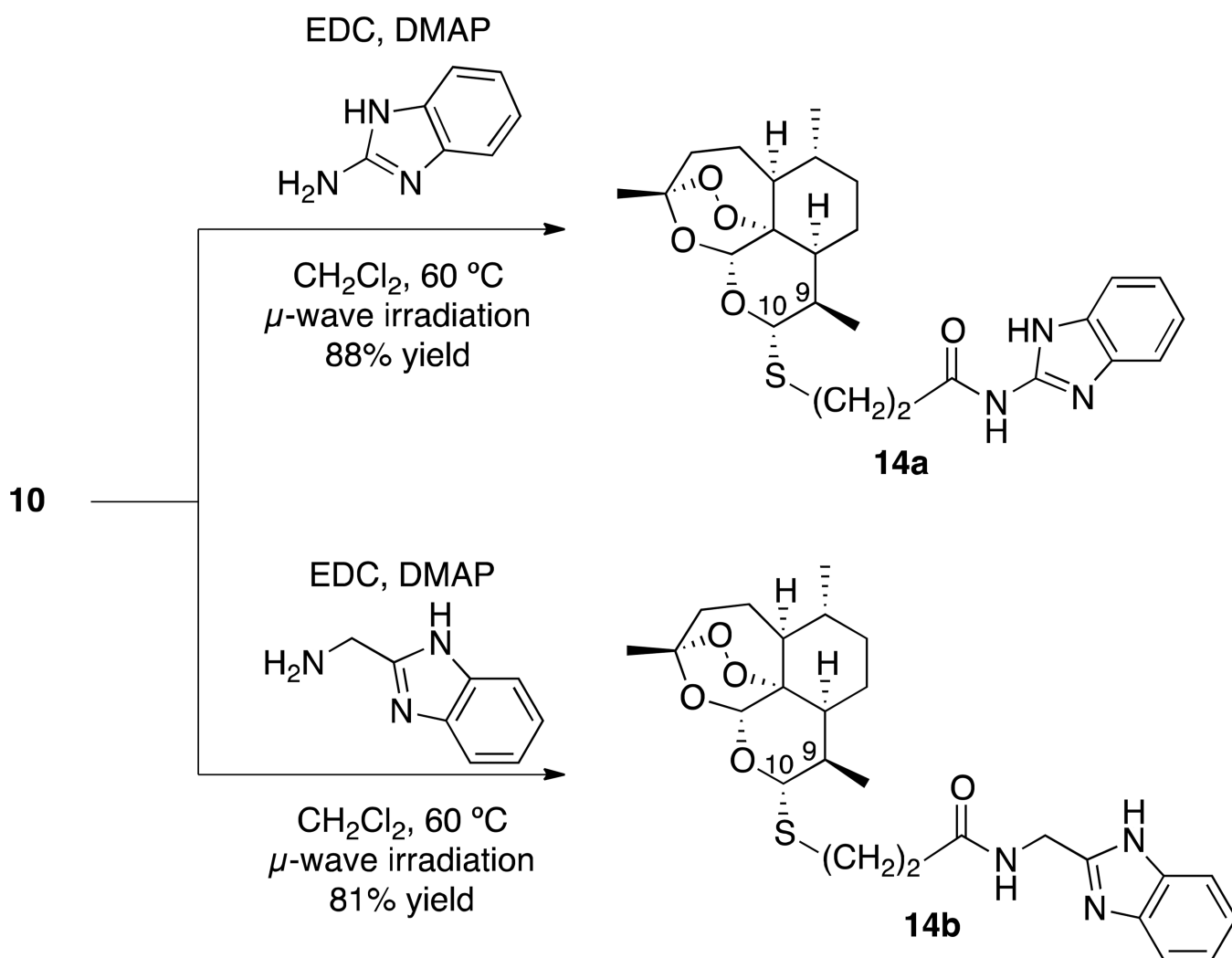
Scheme 3.

Scheme 4.

Scheme 5.
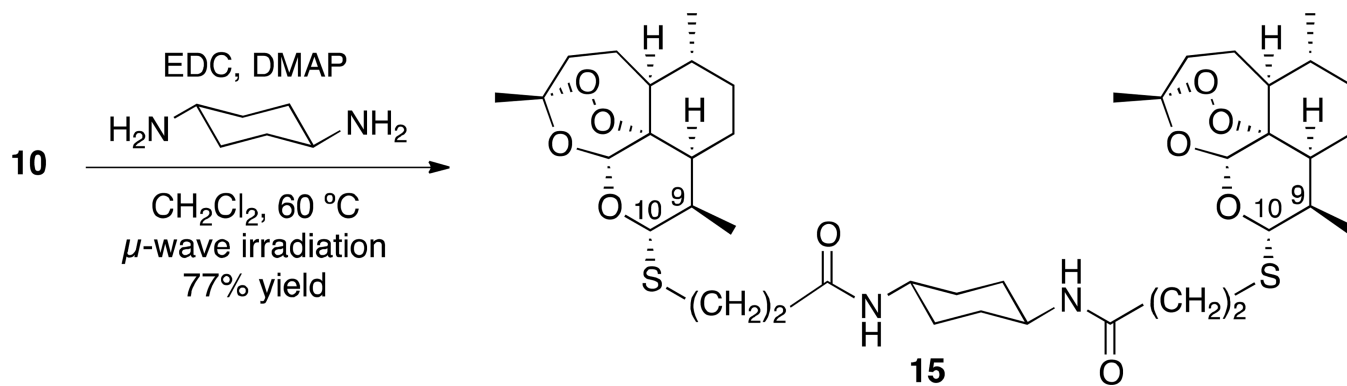
Scheme 6.

Table 1

C-10 Thioacetal Carbonates **11**

Compound	n	R	log P [a]	Yield
11a	3	Me	4.6	91%
11b	3	*n*-Bu	5.9	81%
11c	3	*t*-Bu	5.5	85%
11d	3	PhF-4	6.4	77%
11e	3	CH$_2$C≡CH	4.9	54%
11f	6	Me	5.9	82%
11g	6	Et	6.3	88%
11h	6	CH$_2$C≡CH	6.1	64%
11i	6	O(CH$_2$)$_2$S(O)$_2$Ph	6.6	63% [b]

[a]Log P values were calculated using ChemOffice® Ultra 11.0

[b]This reaction was run in dichloromethane with pyridine as the base

Table 2

C-10 Thioacetal Thiocarbonates **12**

Compound	X	R	log P [a]	Yield
12a	O	Me	5.2	81%
12b	O	*n*-Pr	6.0	77%
12c	O	*t*-Bu	6.1	82%
12d	S	Me	5.6	70%

[a]Log P values were calculated using ChemOffice® Ultra 11.0

Table 3

Calculated log P values for C-10 thioacetals **13**–**15**

Compound	log P[a]
13a	4.7
13b	5.3
14a	4.8
14b	4.5
15	7.4

[a]Log P values were calculated using MarvinSketch and a calculator plug-in by ChemAxon Kft

Table 4

In vivo antimalarial efficacy using a single oral dose of 6 mg/kg trioxane and 18 mg/kg mefloquine hydrochloride in *P. berghei*-infected mice.

Trioxane	Average survival (days) after infection	% Suppression of parasitemia (on day 3 post infection)
11a	12.8 (13, 13, 13, 12)	>99.9%
11b	18.5 (30, 16, 15, 13)	>99.9%
11c	17.8 (28, 17, 13, 13)	>99.9%
11d	19.3 (29, 17, 17, 14)	>99.9%
11e	18.8 (30, 17, 15, 13)	99.9%
11f	21.8 (30, 30, 15, 12)	>99.9%
11g	16.5 (28, 14, 12, 12)	>99.9%
11h	**25.3** (30, 29, 27, 15)	>99.9%
11i	14.5 (17, 15, 13, 13)	99.9%
12a	**24.5** (30, 30, 21, 17)	>99.9%
12b	19.3 (30, 17, 15, 15)	>99.9%
12c	**29.8** (30, 30, 30, 29)	99.9%
12d	22.0 (30, 28, 17, 13)	99.9%
13a	23.0 (30, 29, 17, 16)	>99.9%
13b	22.3 (30, 29, 17, 13)	>99.9%
14a	**26.3** (29, 28, 27, 21)	>99.9%
14b	19.5 (29, 20, 15, 14)	>99.9%
15	19.8 (30, 17, 17, 15)	99.9%
Controls		
Infected (no drug)	8.0 (10, 8, 7, 7)	0%
Artemether + mefloquine-HCl	16.5 (28, 13, 13, 12)	>99.9%
Mefloquine-HCl only	14.0 (17, 13, 13, 13)	>99.9%