New Chimeric Antimalarials with 4-Aminoquinoline Moiety Linked to a Tetraoxane Skeleton[1]

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The synthesis of the chimeric molecules consisting of two pharmacophores, tetraoxane and 7-chloro-4-aminoquinoline, is reported. The tetraoxanes **2**, **4**, and **8** show relatively potent in vitro antimalarial activities, with IC_{90} values for the *Plasmodium falciparum* strain W2 of 2.26, 12.44, and 10.74 nM, respectively. In addition, two compounds, **2** and **4**, cured mice in a modified Thompson test for antimalarial blood stage activity, with a minimum curative dose of 80 mg/kg, a minimum active dose of 20 mg/kg/day, and a maximum tolerated dose of >960 mg/kg.

Introduction

New therapies are urgently needed to treat the devastating tropical disease, malaria, which is caused by the protozoan parasite, *Plasmodium falciparum*, and results in 300−500 million people annually becoming ill from the disease with over 1.5 million of these cases resulting in death.[2] The development of widespread drug-resistance to chloroquine (CQ[a]), a standard antimalarial drug, has resulted in severe health issues for countries in malaria endemic regions. The antimalarial properties of artemisinin[3] and of other peroxides, such as 1,2,4,5-tetraoxacycloalkanes (tetraoxanes),[4] are currently being investigated as new approaches to fighting CQ-resistant strains of malaria. In addition, it was reported that trioxane−aminoquinoline chimeras ("trioxaquines")[5] and artemisinin−quinine hybrids[6] possess improved antimalarial activity in comparison to parent drugs. Recently, acridine−endoperoxide derivatives used for a mechanistic study were shown to exert low nanomolar in vitro activities.[7]

The tetraoxanes appear to lack cytotoxic effects in vitro and appear to be safe in vivo. The results of an earlier study[4] indicate that the compounds demonstrate relatively low cytotoxicity against healthy cells (PBMC, VERO) as compared to their antimalarial activity (SI = 826−33000).[8] In addition, tetraoxanes tested for in vivo efficacy exerted no apparent toxic effect on test animals even upon necropsy (MTD >1800−960 mg/kg).[4i,k]

One part of our research in this field is focused on the development of a new type of tetraoxane that is composed of nonidentical substituents that utilize a steroid and small cyclohexylidene carrier possessing secondary amide bonds to enhance their solubility in protic solvents. Accordingly, in this work, we present the results of our study on the preparation and antimalarial activity of tetraoxane compounds possessing a 4-aminoquinoline moiety as the substituent. We endeavored to enhance antimalarial activity of the tetraoxane moiety,[9] which is known to act as radical donor,[4k] by introducing into the molecule a second pharmacophore that is an aminoquinoline entity known to interfere with hematin polymerization.[10] We also hypothesized that this chimeric synthetic approach should facilitate penetration of the tetraoxanes into infected erythrocytes[10] on the basis of several lines of evidence. For example, our very recent findings indicate that tetraoxane−amines exert very good in vitro (nanomolar) and in vivo activities (MCD ≤ 37.5 mg/kg/day)[11] and that the tetraoxane moiety is relatively stable under acidic conditions (stable at pH 1.6).[4k] Furthermore, the results of in vitro metabolic stability tests[4i] suggest that the ethandiaminoaminoquinoline entity, in addition to being the hematin ligating agent, is likely to act as a base that could be protonated, thus increasing the concentration of tetraoxanes within the food vacuole.

Accordingly, in this work, we present the results of our study on the preparation and antimalarial activity of chimeric tetraoxanes. We also describe initial results of antimycobacterial screening, supporting an additional therapeutic indication for these chimeric molecules.

Chemistry. The amides **2** and **8** were prepared from tetraoxanes **1**[11] and **7**,[4h] respectively, using a mixed anhydride method, whereas for the synthesis of tetraoxane-amines **4** and **10**, we exploited our recent findings on the stability of the tetraoxane moiety to reductive amination and oxidation conditions (Scheme 1).[11]

Thus, the tetraoxane−alcohol **5**[11] was oxidized with PCC in high yield, and the obtained aldehyde **6** was reductively coupled to aminoquinoline AQ, affording compound **4** in 55% overall yield. In analogy, the aldehyde **9**[11] underwent reductive amination affording **10** in 70% yield. All compounds were fully characterized using spectroscopic methods.

Antimalarial Activity. The synthesized tetraoxanes **2**, **4**, **8**, and **10** were screened in vitro against three *P. falciparum* strains: D6 (chloroquine-susceptible), W2 (chloroquine-resistant, susceptible to mefloquine), and TM91C235 (a multidrug-resistant strain derived from Thailand),[12] following the protocol given in ref 4e. Of the two types of spirocyclohexylidene tetraoxanes, the amides **2** and **8** and amines **4** and **10**, the former ones exhibit higher in vitro activity against all three *P. falciparum* strains (Table 1). Relative to artemisinin (ART) activity, the amine **10** is less active, while the dicyclohexylidene amine **4** is equipotent. In comparison, the tetraoxane **2**, being more active than its steroidal analogue **8**, is several times more active than ART at IC_{50} and IC_{90} levels.[13] It is interesting to note that these

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[a] Abbreviations: CQ, chloroquine; MFQ, mefloquine; ART, artemisinin; MCD, minimum curative dose; MAD, minimum active dose; PCC, pyridinium chlorochromate.

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Table 1. In Vitro Antimalarial Activities of Tetraoxanes **2**, **4**, **8**, and **10** against *P. falciparum* Strains

	IC_{50} (nM)		IC_{90} (nM)			
compd	D6[a]	W2[b]	TM91C235[c]	D6	W2	TM91C235
2	2.33	2.00	3.70	3.75	2.26	7.38
4	9.05	5.76	9.90	18.24	12.44	18.30
8	6.53	4.47	8.54	10.61	10.74	17.20
10	33.45	22.95	30.20	103.47	30.29	159.91
MFQ[d]	19.01	5.35	47.47	44.76	18.58	152.81
CQ[d]	12.08	421.59	129.78	15.94	639.71	263.56
ART[e]	9.0	6.7	13.04	12.8	11.5	17.40

[a] *P. falciparum* African D6 clone. [b] *P. falciparum* Indochina W2 clone. [c] Multidrug *P. falciparum* Thailand TM91C235 clone. [d] Control compounds. [e] Average of greater than eight replicates.

synthesized compounds are most active against the CQ-resistant W2 strain, and the IC_{90} TM91C235/D6 ratio falls within 1 and 2, demonstrating a similar parasite strain susceptibility profile as ART. Compound **2** showed the most potent in vitro antimalarial activity in this series because it was five times more active against D6 than CQ, ca. five times more active than ART against W2, and more than two times more active than ART against the multidrug resistant TM91C235 strain.

The oral activities of compounds **2** and **4** were tested against *P. berghei* (KBG 173 strain) infected ICR mice using a modified Thompson test at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Thailand.[4i] Mice were infected on day 0, and the tested compounds were administered on days 3−5 postinfection. Both compounds cured five out of five mice at the highest tested dose of 320 mg/kg/day (Table 2), and some mice were cured also at 80 mg/kg/day. Results indicate a minimum curative dose (MCD) of 80 mg/kg/day and minimum active dose (MAD) of 20 mg/kg/day for both compounds. At the dosage of 320 mg/kg/day for compounds **2** and **4**, all mice survived through day 31, as evidenced by negative blood smears from days 6−31 with no significant gross lesions upon necropsy. At 80 mg/kg/day, all mice were negative the day after the final treatment dose (day 6) for both compounds. Compound **2** at 80 mg/kg/day completely cured 1/5 mice, whereas suppression of parasitemia followed by self-cure was observed (parasite recrudescence) in 4/5. Compound **4** was more active because it afforded complete cure in 3/5 mice, with recrudescence occurring in 2/5. An observation of no toxic effects upon necropsy even at the highest dose of 960 mg/kg for three consecutive days adds to our earlier observations of perceivable safety for our tetraoxanes.

The in vitro metabolism studies were performed using the most in vitro active compound **2** to assess its bioavailability as a drug candidate with oral administration. Metabolic stability assays were done using human and mouse liver microsomes.[4i] The data showed that compound **2** was relatively metabolically stable, with a half-life >60 min in both mouse and human liver microsomes.[14] It is of interest to note that the tetraoxane functionality itself is metabolically stable, indicating that the compounds tested, and their reductive scission products (upon exerting lethal effect on the parasite), were nontoxic to experimental animals.

In addition, tetraoxanes **2**, **4**, **8**, and **10** were tested against *Mycobacterium tuberculosis*, strain H37Rv, at TAACF.[15] As for preliminary results, all four compounds were considered active at the cutoff concentrations of 6.26 μg/mL, (>90% inhibition, Alamar blue assay). For compounds **4**, **8**, and **10**, the inhibitory activities were determined in the MABA assay (IC_{90} = <0.43, 0.40, and 0.33 μM, respectively) and, again, all three were considered active.

We are the first to report the synthesis and antimicrobial activities of chimeric molecules consisting of two pharmacophores, tetraoxane, and 7-chloro-4-aminoquinoline, which we shall refer to as "tetraoxaquines". The results obtained indicate that, like with trioxaquines, the aminoquinoline part of the molecule did not contribute extensively to in vivo antimalarial activity in comparison to previously synthesized tetraoxane−amines.[11] However, the excellent in vitro activity of compound **2** against CQ-resistant and CQ-susceptible *P. falciparum* strains, and satisfactory initial antituberculosis data for **4**, **8**, and **10**, validates the synthetic efforts toward more active analogues.

Experimental Section

For general remarks see ref 4i.

***N*-2-[(7-Chloroquinolin-4-yl)amino]ethyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxamide (2).** A solution of **1**[16] (100 mg, 0.37 mmol), Et_3N (52 μL, 0.37 mmol), and $ClCO_2Et$ (34.8 μL, 0.37 mmol) in dry CH_2Cl_2 (10 mL), was stirred for 90 min at 0 °C. Then, the amine AQ[17] (163 mg, 0.73 mmol) was added, and after 30 min of stirring, the reaction mixture was warmed to rt. After an additional 90 min, it was diluted with H_2O, the layers were separated, and the organic layer was washed with brine, dried over anhyd $MgSO_4$, and evaporated to dryness. Upon dry-flash purification, the amide **2** (148 mg, 85%); mp = 193−200 °C (MeOH). Anal. ($C_{24}H_{30}ClN_3O_5 \cdot H_2O$) C, H, N.

***N*¹-(7-Chloroquinolin-4-yl)-*N*²-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-ylmethyl)-1,2-ethandiamine (4). Procedure A.** A solution of **3**[11] (106 mg, 0.4 mmol) and bromide AQ-Br[18] (118 mg, 0.4 mmol) in DMF (7 mL), with added Et_3N (58 μL, 0.4 mmol), was stirred for 120 h at room temperature. Reaction mixture was diluted with EtOAc and H_2O, and the layers were separated. The water layer was further extracted with EtOAc (2 × 50 mL), combined organic layers were dried over anhyd $MgSO_4$ and evaporated to dryness. The crude product **4** was purified using dry-flash chromatography, eluent EtOAc/MeOH/NH_{3aq} (8/1/1). Yield 104 mg (55%). Colorless foam softness at 147−151 °C. Anal. ($C_{24}H_{32}ClN_3O_4 \cdot 0.5H_2O$) C, H, N.

Procedure B. To a solution of alcohol **5**[11] (510 mg, 1.97 mmol) in dry CH_2Cl_2 (20 mL), PCC (638 mg, 2.96 mmol) was added, and the resulting suspension was stirred for 2 h at room temperature. The crude product was purified using dry-flash chromatography, eluent CH_2Cl_2. Such obtained aldehyde (yield 440 mg; 86%) was used immediately thereafter. To a mixture of aldehyde (440 mg, 1.7 mmol) and amine AQ (380 mg, 1.7 mmol) in CH_2Cl_2 (20 mL), sodium triacetoxyborohydride (728 mg, 3.4 mmol) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water and extracted with CH_2Cl_2 (2 × 50 mL). Combined organic layers were dried over anhyd Na_2SO_4 and evaporated to dryness. The crude product was purified by dry-flash chromatography, eluent EtOAc/MeOH/NH_{3aq} = 8/1/1. Yield 505 mg (64%).

***N*-2-[(7-Chloroquinolin-4-yl)amino]ethyl-7α,12α-diacetoxy-5β-cholan-24-amido-3-spiro-6′-(1′,2′,4′,5′-tetraoxacyclohexane)-3′-spirocyclohexane (8).** The acid **7**[4h] (200 mg, 0.32 mmol) was transformed into amide **8** (212 mg 80%) using amine AQ (2 equiv, 143 mg, 0.64 mmol) in dry CH_2Cl_2 (10 mL). The crude product was purified by column chromatography Lobar B, LichroPrep RP-18, eluent MeOH/H_2O = 9/1. Colorless foam, softness at 142−145 °C. $[\alpha]_D^{20}$ = +41.0 (c = 0.2, $CHCl_3$). Anal. ($C_{45}H_{62}ClN_3O_9 \cdot 3H_2O$) C, H, N.

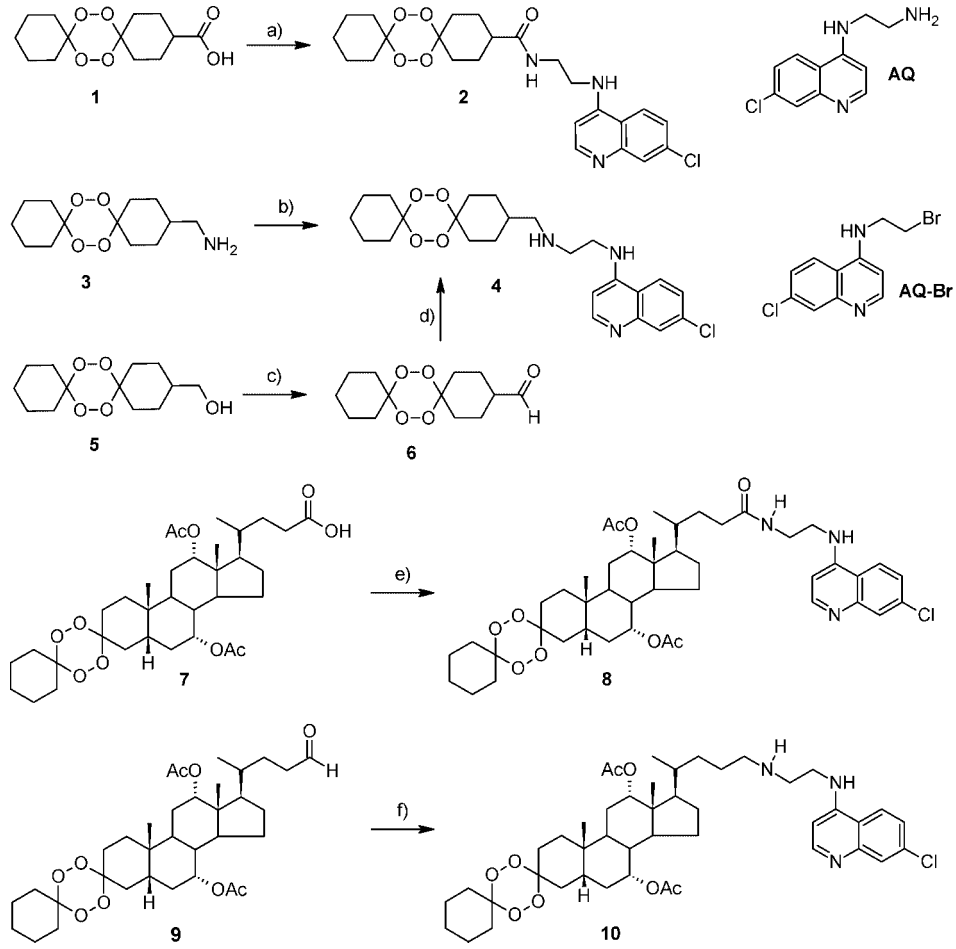
***N*¹-(7-Chloroquinolin-4-yl)-*N*²-(7α,12α- diacetoxy-5β-cholan-24-amino-3-spiro-6′-(1′,2′,4′,5′- tetraoxacyclohexane)-3′-spirocyclohexane)-1,2-etandiamine (10).** Aldehyde **9**[11] (200 mg, 0.33 mmol) was transformed into amine **10** (187 mg, 70%) using amine AQ (147 mg, 0.66 mmol) and $NaBH(OAc)_3$ (140 mg, 0.66 mmol). The crude product was purified using dry-flash chromatography, eluent EtOAc/MeOH (1/1). Colorless foam, softness 102−105 °C. $[\alpha]_D^{20}$ = +42.5 (c = 0.2, $CHCl_3$). Anal. ($C_{45}H_{64}ClN_3O_8 \cdot 0.5H_2O$) C, H, N.

Table 2. In Vivo Antimalarial Activities of Tetraoxanes **2** and **4** against *P. berghei*

compd	mg/kg/day	mice dead/ day died	mice alive on day 31/total	survival time (day)[b]
2[a]	320		5/5	>31
	80	1/18, 1/20	3/5	26.2
	20	1/10, 1/12, 1/13, 1/17, 1/19	0/5	14.2
4[a]	320		5/5	>31
	80	1/12, 1/27	3/5	26.4
	20	2/14, 3/17	0/5	15.8
infected controls[c]	0	6−8	0/5	

[a] Groups of five *P. berghei* (KBG 173 strain) infected ICR mice were treated po once per day on days on 3−5 postinfection with tetraoxanes suspended in 0.5% hydroxyethylcellulose−0.1% Tween 80. Mice alive on day 31 with no parasites in a blood film are considered cured. [b] Including cured mice. [c] All noninfected age controls survived (5/5).

Scheme 1[a]



[a] (i) $ClCO_2Et$, Et_3N; (ii) AQ, CH_2Cl_2 85%. (b) AQ-Br, DMF/Et_3N, 55%. (c) PCC, CH_2Cl_2, 86%. (d) AQ, $NaBH(OAc)_3$, CH_2Cl_2, 64%. (e) (i) $ClCO_2Et$, Et_3N; (ii) AQ, CH_2Cl_2, 80%. (f) AQ, $NaBH(OAc)_3$, CH_2Cl_2, 70%.

In Vitro Antimalarial Activity. The in vitro antimalarial drug susceptibility screen is a modification of the procedures first published by Desjardins et al.,[19] with modifications developed by Milhous et al.,[20] and the details are given in ref 4e.

In Vivo Antimalarial Activity. The *P. berghei* mouse efficacy tests were conducted using a modified version of the Thompson test. Briefly, groups of five mice were inoculated intraperitoneally with erythrocytes infected with *P. berghei* on day 0. Drugs were suspended in 0.5% hydroxyethylcellulose−0.1% Tween 80 and administered orally once a day beginning on day 3 post infection. Dosings are given in Table 2. Untreated control mice lethally succumb to infection on day 6−8 postinfection. Cure was defined as survival until day 31 post-treatment.

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Supporting Information Available: Analytical data of synthesized compounds. This material is free of charge via the Internet at http://pubs.acs.org.

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(13) IC_{90} can be envisaged to represent a suitable compromise between the substantial amount of data obtainable with IC_{50} (value based on a relatively high number of parasites, but with less relevance to the therapeutic situation) and IC_{99} (reflects MIC and full efficacy, however, based on a relatively small number of surviving parasites available for analysis).

(14) Stable compounds were defined as having half-lives of >60 min. Experimental details on metabolic stability studies are given in ref 11.

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