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A novel artemisinin–quinine hybrid with potent antimalarial activity

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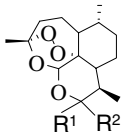
Abstract—Artemisinin was reduced to dihydroartemisinin and coupled to a carboxylic acid derivative of quinine via an ester linkage. This novel hybrid molecule had potent activity against the 3D7 and (drug-resistant) FcB1 strains of *Plasmodium falciparum* in culture. The activity was superior to that of artemisinin alone, quinine alone, or a 1:1 mixture of artemisinin and quinine.

Malaria is a devastating disease, with an annual mortality rate of over one million. The protozoal parasite responsible, *Plasmodium falciparum*, has gained resistance to most forms of monotherapy especially in Southeast Asia, South America and East Africa.[1] For this reason, the use of combination chemotherapy that incorporates the use of a fast acting antimalarial agent of the artemisinin family **1**–**5** with other antimalarials like quinine[2] or lumefantrine[3] is now recommended. Combinations of artemisinins, which kill parasites rapidly but are also rapidly excreted, with longer half-life antimalarial agents are favoured in order to achieve full eradication of parasites and prevent the recrudescence commonly found with artemisinin monotherapy.

The artemisinin group of compounds includes artemisinin **1**, isolated from *Artemisia annua*, and its semisynthetic derivatives, the reduced lactol, dihydroartemisinin **2**, the oil-soluble artemether **3** and arteether **4** and the water-soluble derivative artesunate **5**,[4] all of which are effective against both asexual and sexual blood-stage parasites. Their mode of action is mediated by a unique structural component, the endoperoxide bridge. The target is controversial but recent evidence suggests that an Fe^{2+}-activated form of the drug potently inhibits PfATP6, a key parasite Ca^{2+} transporter.[5]

Quinine **6** is still the drug of choice for the treatment of severe malaria and has been since the introduction of *Cinchona* bark to European medicine in the 1630s.[6] It is effective against the asexual erythrocytic forms of malaria, possibly as a result of interference with host haemoglobin digestion.[4]



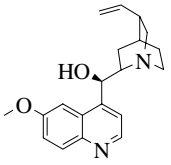
R^1, R^2 = O = artemisinin, **1**

R^1 = H, R^2 = OH = dihydroartemisinin, **2**

R^1 = H, R^2 = OMe = artemether, **3**

R^1 = H, R^2 = OEt = arteether, **4**

R^1, = H, R^2 =$OCH_2CH_2CO_2H$ = artesunate, **5**



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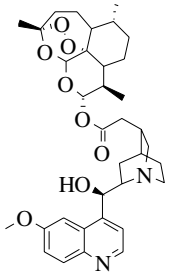
In view of this background and of the reported antimalarial synergism between artemisinins/other endoperoxides and quinine,[7] this article reports on the first example of a covalently linked artemisinin–quinine hybrid **7** in which the vinyl functionality of quinine was modified to allow for the attachment of dihydroartemisinin.



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Keywords: Malaria; Artemisinin; Quinine; *Plasmodium falciparum*; Resistance; Combination chemotherapy.
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Hybrid molecules in which the two compounds are linked offer a simpler and possibly more effective way to deliver these agents. With respect to artemisinin, modification to the lactone functionality is well tolerated with derivatives **2**–**5** all possessing potent antimalarial activity.[8] As dihydroartemisinin **2**, containing the easily esterifiable hemiacetal functionality, is one of the principal artemisinin metabolites formed in vivo, it was selected as the most appropriate artemisinin derivative to form the artemisinin–quinine hybrid. The decision to modify the vinyl functionality of quinine was based on previous studies with this compound which indicated that modification to other potential sites had unfavourable effects on activity. In particular, the hydroxyl group and the quinoline ring are essential for activity but the quinuclidine ring can be substantially modified without loss of activity. Alterations to the stereochemical centres on quinine have mixed effects with erythro configurations at the C-8 and C-9 positions of quinine analogues being more active than the threo isomers for some, but not all, derivatives.[9]

The first steps in the synthesis of the artemisinin–quinine hybrid **7** involved the conversion of the alkene functionality of quinine into its carboxylic acid derivative **9**. The method chosen followed that of a literature procedure.[10] In brief, the hydroxy functionality of quinine **6** was protected as a *tert*-butyldimethylsilyl (TBDMS) ether, using Et_3N, DMAP and TBDMSCl. Conversion of the vinyl functionality of TBDMS quinine **8** to the carboxylic acid derivative **9** involved a hydroboration step with BH_3/THF in diglyme and cleavage of the borane complex with $Me_3NO{\cdot}2H_2O$ to yield the primary alcohol which following oxidation with Jones's reagent afforded the acid **9**. Dihydroartemisinin was obtained following reduction of artemisinin with $NaBH_4$ in methanol and was coupled to **9** using 2,6-dichlorobenzoyl chloride as coupling reagent, Et_3N as base and DMAP as acylation catalyst[11]. Removal of the TBDMS group from **10** was accomplished following treatment with TBAF in THF.[11] Only the most significant isomer of the hybrid was obtained following purification by preparative TLC. From the ^1H NMR spectrum, the isomer isolated was the alpha isomer **7** as the large coupling constant for H-10 at δ 5.74, $J = 10.0$ Hz, is indicative of a *trans* diaxial relationship between H-10 and H-9, whereas for a gauche relationship to exist between these protons the coupling constant would be expected to be of the order of 3–4 Hz.[12] Utilizing a combination of ^1H, ^{13}C, HH, HC and HMBC COSY spectra, all of the proton and carbon signals on the artemisinin–quinine hybrid **7** were assigned.[11]

We confirmed that this compound has superior activity to that of artemisinin alone, quinine alone, or a 1:1 mixture of artemisinin and quinine. The artemisinin–quinine hybrid had potent antimalarial activity in culture (see Table 1). *P. falciparum* 3D7 was inhibited by much lower concentrations of the hybrid than of quinine or artemisinin alone, suggesting that the actions of both quinine and artemisinin moieties were preserved. Moreover, when the activity of the hybrid was compared with that of a 1:1 mixture of quinine and artemisinin (on a mol quinine/mol artemisinin basis), the hybrid was about threefold superior. This suggested that the two molecules joined together were more active than the same two molecules administered separately. The higher activity of the hybrid may however be the result of its cleavage to form quinine and dihydroartemisinin, the latter compound being more active than artemisinin itself.[13] Similar results were obtained with the chloroquine-resistant strain FcB1 (Table 1). Note that the antimalarial activities were determined after both 48 and 72 h of incubation because of the relatively slow action of quinine.

The reported results demonstrate a proof of concept for the linkage of artemisinin and quinine in a single molecule that retains and possibly enhances the antimalarial activity of the parent compounds. Given the metabolic lability of the ester linkage it is uncertain whether the link would be preserved in vivo but the general synthetic approach could also be used for both ether and amide linkages. Hybrids of synthetic, artemisinin-like trioxanes and chloroquine[14,15] and of trifluoromethylartemisinin and mefloquine[16] have also been shown to possess antimalarial activity. Such hybrids may be more effective in some respects than fixed-ratio combinations of the individual drugs. This compound is expected to readily form soluble salts in the same way as quinine so may offer an

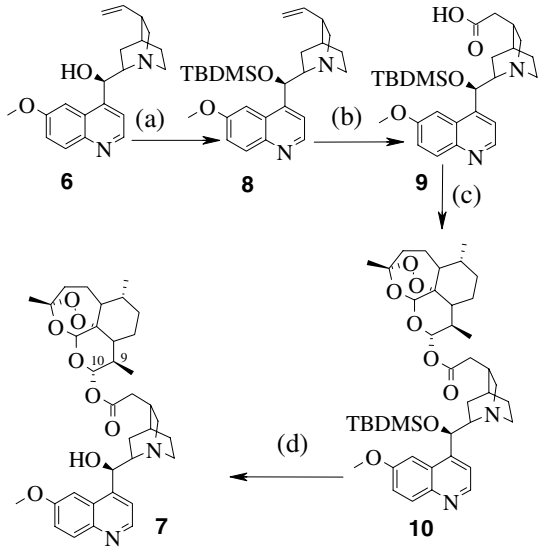
Table 1. Fifty percent inhibitory concentrations (IC_{50}) of the artemisinin–quinine hybrid compared with the individual drugs

Compound	3D7 (48 h)	3D7 (72 h)	FcB1 (48 h)	FcB1 (72 h)
Geometric mean IC_{50}/nM (95% confidence limits)				
Quinine	149 (95.1, 232)	73.5 (57.0, 94.6)	96.8 (74.5, 126)	75.3 (59.0, 96.1)
Artemisinin	49.4 (40.7, 60.0)	45.5 (35.3, 58.6)	50.0 (43.7, 57.3)	55.0 (39.0, 77.4)
Art-Qui-OH	8.95 (6.59, 12.2)	10.4 (6.06, 17.9)	9.59 (7.06, 13.0)	10.2 (4.73, 21.9)
Quinine + artemisinin[a,b]	31.8 (27.4, 37.0)	28.6 (21.5, 38.2)	27.9 (26.5, 29.5)	26.3 (24.7, 28.0)

Activities against cultured, asychronous, blood-stage *P. falciparum* strains 3D7 and FcB1 were determined after 48 and 72 h using the parasite lactate dehydrogenase assay as previously described.[17,18] Dose–response curves were used to determine the IC_{50} and the results are expressed as geometric means of IC_{50} from three duplicate determinations.

[a] Values represent concentrations of each of quinine and artemisinin in a 1:1 ratio, for example, a combination of 31.8 nM quinine + 31.8 nM artemisinin inhibited the growth of 3D7 by 50% after 48 h.

[b] Difference from Art-Qui-OH (artemisinin–quinine hybrid) by Student's *t* test: $p = 0.0001$ (3D7, 48 h), 0.015 (3D7, 72 h), 0.0011 (FcB1, 48 h), 0.056 (FcB1, 72 h).



Scheme 1. Reagents and conditions: (a) TBDMSCl, Et_3N, DMAP, DMF, rt; (b) i—BH_3–THF, diglyme, 0 °C; ii—$Me_3NO{\cdot}2H_2O$, 100 °C; iii—Jones reagent, acetone, rt; (c) i—2,6-dichlorobenzoyl chloride, dihydroartemisinin, Et_3N, DMAP, DCM, rt; (d) TBAF, THF, rt.

improvement in formulation and allow for shorter treatment with enhanced compliance. The increased potency of the hybrid may arise through its enhanced cellular uptake over that of the individual components. Additionally, this new hybrid may act as a 'mutual prodrug' in the case where the ester group is hydrolysed to the individual components and thus act as a unique way of delivering these antimalarial agents to the protozoal site of action. Also the combination might be expected to show a decrease in the duration of the side effects that are often associated with quinine regimens and additionally offer the possibility of a new antimalarial drug with discrete activity in its own right (Scheme 1).

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References and notes

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11. To a stirred solution of the acid **9** (50 mg, 0.1 mmol) in DCM at room temperature were added Et_3N (20 mg, 0.2 mmol), DMAP (48.8 mg, 0.4 mmol), 2,6-dichlorobenzoyl chloride (22.8 mg, 0.12 mmol) and dihydroartemisinin **2** (34 mg, 0.12 mmol). After 2 h, the reaction was quenched following the addition of water and the mixture extracted with DCM (3× 50 ml). The combined organic layers were dried with Na_2SO_4, filtered and solvents were removed by evaporation under reduced pressure to yield an oily residue which was purified by flash column chromatography eluting initially with pure EtOAc then steadily adding MeOH to a maximum of 20% to yield the protected artemisinin–quinine hybrid **10** as a viscous oil (30 mg, 39.9%). The resulting oil (20 mg, 0.027 mmol) was dissolved in a solution of TBAF in THF (100 μl, 1 M in THF) and THF (1.5 ml) and allowed to stir at room temperature for 2 h. The reaction was quenched by the addition of water, and the mixture extracted with DCM (2× 20 ml). The combined organic layers were dried over Na_2SO_4, filtered and reduced in volume to yield an oily residue. Following purification by preparative TLC, the artemisinin–quinine hybrid **7** was isolated (10 mg, 60%) as a white crystalline solid with the following physical characteristics. Decomposition point 120–123 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 8.73 (d, J = 4.5 Hz, 1H, NC*H*CHCC), 7.93 (d, J = 9 Hz, 1H, CHC(OCH$_3$)CHC*H*C), 7.60 (d, J = 4.5 Hz, 1H, NCHC*H*CC), 7.26 (d, J = 9 Hz, 1H, CHC(OCH$_3$)C*H*CHC), 7.12 (s, 1H, C*H*C(OCH$_3$)CHCHC), 5.99 (br s, 1H, OOC*H*(OH)C), 5.72 (d, J = 10.0 Hz, 1H, OOC*H*CH(CH$_3$)CHCH$_2$CH$_2$), 5.40 (s, 1H, OOCHC), 3.83 (s, 3H, OMe), 3.21 (m, 1H, NCH$_2$CH$_2$CHCH$_2$C*H*), 3.98, 2.88 (2 × m, 2H, NC*H*$_2$CH$_2$CHCH$_2$CH), 3.36, 2.64 (2 × m, 2H, NC*H*$_2$CHCH$_2$CO), 2.48 (m, 1H, NCH$_2$C*H*CH$_2$CO), 2.47 (m, 1H, OOCHC*H*(CH$_3$)CHCH$_2$CH$_2$), 2.41 (m, 2H, NCH$_2$CHC*H*$_2$CO), 2.0, 2.41 (2 × m, 2H, CH$_3$CHCHCH$_2$C*H*$_2$CCH$_3$), 2.02 (m, 1H, NCH$_2$CH$_2$C*H*CH$_2$CH), 1.56 (m, 1H, OOCHCH(CH$_3$)C*H*CH$_2$CH$_2$), 1.91, 1.49 (2 × m, 2H, NCH$_2$CH$_2$CHC*H*$_2$CH), 1.40 (s, 3H, CH$_3$CHCHCH$_2$CH$_2$CC*H*$_3$), 1.79, 1.39 (2 × m, 2H, NCH$_2$C*H*$_2$CHCH$_2$CH), 1.35 (m, 1H, CH$_3$C*H*CHCH$_2$CH$_2$CCH$_3$), 1.25 (m, 2H, CH$_3$CHCHC*H*$_2$CH$_2$CCH$_3$), 1.25 (m, 1H, CH$_3$CHC*H*CH$_2$CH$_2$CCH$_3$), 1.25 (m, 2H, OOCHCH(CH$_3$)CHC*H*$_2$CH$_2$), 1.75, 1.02 (2 × m, 2H, OOCHCH(CH$_3$)CHCH$_2$C*H*$_2$), 0.95 (d, J = 5 Hz, 3H, C*H*$_3$CHCHCH$_2$CH$_2$CCH$_3$), 0.78 (d, J = 7 Hz, 3H, OOCHCH(C*H*$_3$)CHCH$_2$CH$_2$), ^{13}C (100 MHz, $CDCl_3$, 25 °C): δ 170.2 (NCH$_2$CHCH$_2$*C*O), 158.3 (CH*C*(OCH$_3$)CHCHC), 147.2 (N*C*HCHCC), 145.6 (NCHCH*C*C), 144.1 (N*C*CHCH), 131.6 (CHC(OCH$_3$)CH*C*HC), 125.7 (NCHCHC*C*), 122.2 (CHC(OCH$_3$)*C*HCHC), 118.6 (NCH*C*HCC), 104.0 (CH$_3$CHCHCH$_2$CH$_2$*C*CH$_3$), 100.1 (*C*HC(OCH$_3$)CHCHC), 92.3 (OO*C*HCH(CH$_3$)CHCH$_2$CH$_2$), 91.4 (OO*C*HC), 79.9 (OOCH*C*), 69.1 (*C*HOH, weak signal), 56.8 (NCH$_2$CHCH$_2$CO), 56.5 (CHC(O*C*H$_3$)CHCHC), 51.6 (NCH$_2$CH$_2$CHCH$_2$*C*H), 51.4 (CH$_3$CH*C*HCH$_2$CH$_2$CCH$_3$), 45.1 (OOCHCH(CH$_3$)*C*HCH$_2$*C*H$_2$), 43.4 (N*C*H$_2$CH$_2$CHCH$_2$CH), 38.5 (NCH$_2$CH*C*H$_2$CO), 37.2 (CH$_3$*C*HCHCH$_2$CH$_2$CCH$_3$), 36.1 (CH$_3$CHCHCH$_2$*C*H$_2$CCH$_3$), 34.1 (OOCHCH (CH$_3$)CHCH$_2$*C*H$_2$), 31.8 (OOCH*C*H(CH$_3$)CHCH$_2$CH$_2$), 31.1 (NCH$_2$*C*HCH$_2$CO), 29.9 (OOCHCH(CH$_3$)CH*C*H$_2$ CH$_2$), 29.9 (CH$_3$CHCH*C*H$_2$CH$_2$CCH$_3$), 26.7 (NCH$_2$CH$_2$*C*H CH$_2$CH), 25.6 (CH$_3$CHCHCH$_2$CH$_2$*C*CH$_3$), 24.6 (NCH$_2$ CH$_2$CH*C*H$_2$CH), 22.0 (NCH$_2$*C*H$_2$CHCH$_2$CH), 20.1

($CH_3CHCHCH_2CH_2CCH_3$), 12.7 (OOCHCH(CH_3)CH CH_2CH_2), HRMS MH^+ 623.3331, calculated mass 623.3332.
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