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# Antiplasmodial and antitumor activity of dihydroartemisinin analogs derived via the aza-Michael addition reaction

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ABSTRACT

A series of dihydroartemisinin derivatives were synthesized via an aza-Michael addition reaction to a dihydroartemisinin-based acrylate and were evaluated for antiplasmodial and antitumor activity. The target compounds showed excellent antiplasmodial activity, with dihydroartemisinin derivatives **5**, **7**, **9** and **13** exhibiting $IC_{50}$ values of $\leqslant$10 nM against both D10 and Dd2 strains of *Plasmodium falciparum*. Derivative **4d** was the most active against the HeLa cancer cell line, with an $IC_{50}$ of 0.37 μM and the highest tumor specificity.



Malaria remains the most dangerous and prevalent parasitic disease that occurs in man. Over 200 million episodes of malaria occur annually, occasioning approximately 1 million deaths every year mainly among children and pregnant women in sub-Saharan Africa.[1–4] The main challenge to malaria control is that the malaria parasite, and in particular the more virulent species *Plasmodium falciparum*, has developed clinically significant resistance to many classes of drugs,[5,6] including possibly artemisinin-derived antimalarials.[7,8] This situation has necessitated the current widespread efforts aimed at developing new, highly efficacious drugs to treat malaria.[9]

The antimalarial activities of artemisinins and chloroquine (Fig. 1) have long been recognized and applied clinically.[10–12] However, these compounds have also generated interest as potential precursors for anticancer drug discovery. Artemisinins such as artesunate have been found to be active against a variety of unrelated tumor cell lines, being quite active against leukemia and colon cancer while moderately inhibiting cell growth of lung, breast, ovarian, renal, prostate and CNS cancer cells.[13–15] Other studies have also shown that artemisinins can suppress the growth of human tumor xenografts in rats and mice, possibly by inhibition of angiogenesis.[16–18] On the other hand, chloroquine has been reported as a notable apoptosis-inducing agent against MCF-7 human breast cancer cells, possibly by causing DNA damage and/or suppression of the E2F1 protein.[19] Related studies have revealed the potential anticancer activity of chloroquinolinyl-chalcones against human prostate LNCaP tumor cells.[20]

Our ongoing efforts in the direction of identifying new classes of antimalarial and anticancer agents prompted us to undertake the synthesis and biological evaluation of a variety of antimalarial agents.[21–23] The pharmacophore of chloroquine is generally accepted as the 4-amino-7-chloroquinoline unit[24] (Fig. 1). As such

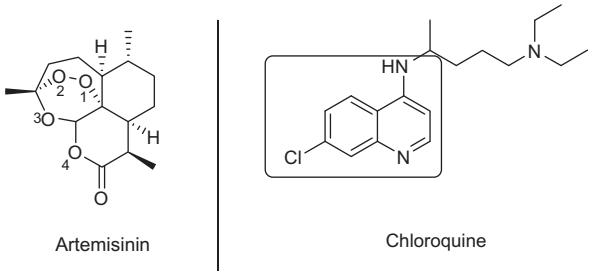


**Figure 1.** Artemisinin and chloroquine (showing the 4-amino-7-chloroquinoline pharmacophore).

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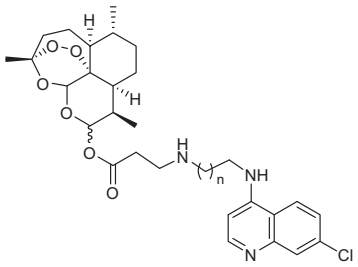
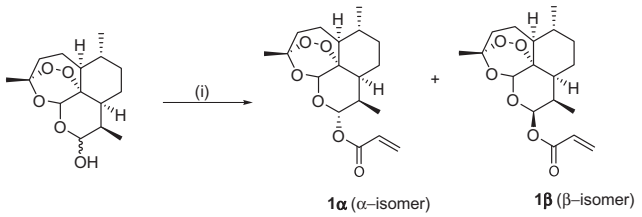


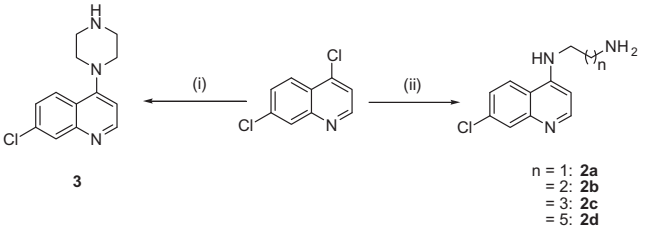
**Figure 2.** General structure of some of the target compounds.



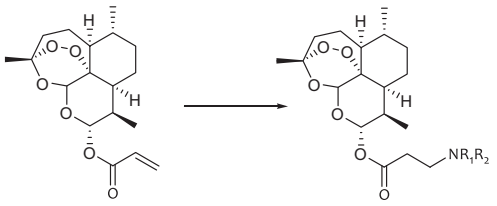

**Scheme 1.** Reagents and conditions: (i) acryloyl chloride (1.2 equiv), $Et_3N$ (1.2 equiv), $CH_2Cl_2$, $N_2$, 0 °C to rt, 12 h.

derivatives **4a**–**d** and **5** were synthesised by employing chloroquinoline amines in the aza-Michael addition reaction (Fig. 2).

The synthetic routes towards the target compounds were simple and straight forward, and commenced with the synthesis of dihydroartemisinin acrylate (**1**). This was synthesized by reacting dihydroartemisinin (DHA) with acryloyl chloride in $CH_2Cl_2$, in the presence of $Et_3N$ (Scheme 1). The requisite acrylate was obtained in 56% yield. $^1H$ NMR of the crude material showed that the product was present as a 1:1 diastereomeric mixture of α- and β-isomers, similar to the starting DHA which was also a 1:1 mixture of α- and β-anomers. Purification by column chromatography yielded the pure stereoisomers.




**Scheme 2.** Reagents and conditions: (i) piperazine (5.0 equiv), $Et_3N$ (1.2 equiv), $K_2CO_3$ (0.5 equiv), NMP, $N_2$, 150 °C, 4 h, 89% yield; (ii) $H_2N(CH_2)_nNH_2$ (20 equiv), 110–150 °C, 4 h, 64–95% yield.



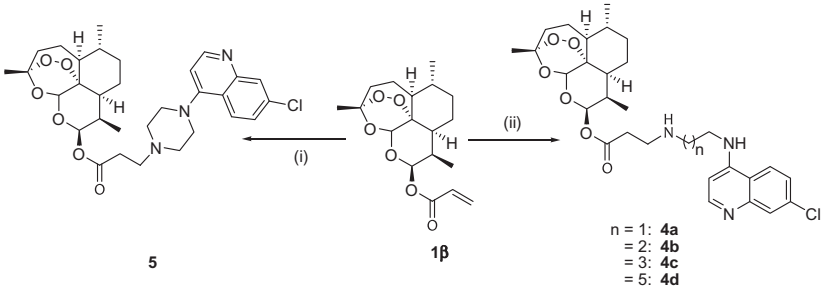

**Scheme 4.** Reagents and conditions: appropriate amine (1.5 equiv), DBU (0.5 equiv), $CH_3CN$, $N_2$, rt, 12 h, 54–80% yield.

It has been reported that the length of the alkyl group between the two nitrogen atoms on the 4-amino side chain may directly influence the degree of biological activity of chloroquine-like compounds. Chloroquine analogs where the alkyl chain between the nitrogen atoms is shortened to 2–4 atoms, or lengthened to 10–12 atoms, retained their activities against chloroquine resistant strains, while those with intermediate chain lengths (5–8 carbon atoms) had rather lower activities.[25] Amines **2a**–**d** (Scheme 2) were therefore chosen such that the number of carbon atoms between the two nitrogen atoms on the side chain spanned across the optimal and intermediate biological activity range (2–6 carbon atoms). The amine with the 5 carbon spacer between the nitrogen atoms was not synthesized as the corresponding starting diamine was not commercially available at the time of synthesis. Compound **3**, incorporating a piperazine moiety at the 4-position, was also synthesized.

Using the protocol described by De et al.,[26] **2a**–**d** were synthesized in moderate to excellent yields from 4,7-dichloroquinoline and an excess (approximately 20 equiv) of the corresponding alkyl diamines (Scheme 2). The large excess of the diamine acted as a base and as solvent, and was also necessary to avoid the formation of terminally di-substituted amines. The synthesis of **3** was achieved by reacting 4,7-dichloroquinoline with an excess of piperazine, using *N*-methylpyrrolidinone (NMP) as the solvent, with $Et_3N$ as the base and a catalytic amount of $K_2CO_3$ (Scheme 2).[27]

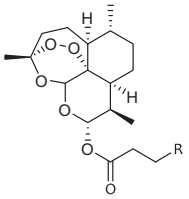
The target compounds were synthesized via the conjugate addition of 7-chloro-4-aminoquinoline amines **2a**–**d** and **3** to the α,β-unsaturated system of DHA acrylate (**1**). Owing to its mildness and operational simplicity, the aza-Michael addition reaction of amines and Michael acceptors has recently attracted much attention as an alternative route for the synthesis of β-aminocarbonyl compounds, of which the target compounds are examples (Fig. 2). Yeom et al.[28] reported a protocol for the aza-Michael addition employing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a promoter, and this mild method was chosen for the synthesis of our target compounds from the DHA acrylate Michael acceptor **1** and the amine intermediates **2a**–**d** and **3** (Scheme 3).

In order to prevent polymerization of the DHA acrylate **1**, 1.5 equiv of the appropriate quinoline amine was used. Additionally, the highly polar *N*,*N*′-dimethylformamide (DMF) was chosen

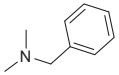
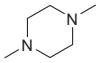
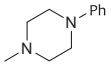



**Scheme 3.** Reagents and conditions: DBU (0.5 equiv), DMF, $N_2$, rt, 12 h; (i) **3** (1.5 equiv), (ii) **2a**–**d** (1.5 equiv) (32–73% yields).

**Table 1**
Results from the in vitro biological evaluation of the intermediates and target compounds



6 - 13

| Compd | C-10 stereochemistry | R | D10 $IC_{50}$ (nM) | Dd2 $IC_{50}$ (nM)) | RI[a] | HeLa $IC_{50}$ (μM) | Primary lymphocytes $IC_{50}$ (μM) | | Tumor specificity |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Resting | PHA-stimulated | |
| **1α** | α | — | 5.85 | 9.27 | 1.6 | 6.37 | 39.58 | 11.47 | 4.0 |
| **1β** | β | — | 7.54 | 12.4 | 1.6 | 2.88 | 30.53 | 9.22 | 6.9 |
| **2a** | — | — | 231.1 | 1142.0 | 4.9 | 7.22 | ND | ND | — |
| **2b** | — | — | 240.3 | 1251.7 | 5.2 | 9.83 | ND | ND | — |
| **2c** | — | — | 482.9 | 2266.4 | 4.7 | 11.24 | ND | ND | — |
| **2d** | — | — | 2164.3 | 1861.4 | 0.9 | 15.69 | ND | ND | — |
| **3** | — | — | 1463.2 | 1639.2 | 1.1 | 27.58 | ND | ND | — |
| **4a** | β | — | 20.6 | 20.1 | 1.0 | 9.47 | >50 | 16.94 | 1.8 |
| **4b** | β | — | 18.7 | 24.4 | 1.3 | 5.58 | 21.52 | 4.71 | 2.4 |
| **4c** | β | — | 24.0 | 27.5 | 1.1 | 10.13 | 32.40 | 8.99 | 2.0 |
| **4d** | β | — | 55.6 | 53.6 | 0.9 | 0.37 | 19.03 | 3.40 | 30.7 |
| **5** | β | — | 10.1 | 10.0 | 1.0 | 6.58 | 44.61 | 14.04 | 4.5 |
| **6** | α |  | 11.3 | 11.4 | 1.0 | ND | ND | ND | - |
| **7** | α |  | 6.23 | 9.02 | 1.4 | 4.80 | >50 | 27.27 | 5.7 |
| **8** | α |  | 13.0 | 13.6 | 1.0 | 6.02 | >50 | 30.45 | 5.0 |
| **9** | α |  | 5.3 | 6.16 | 1.2 | 4.48 | >50 | 25.00 | 5.6 |
| **10** | α |  | 16.9 | 18.7 | 1.1 | 13.04 | ND | ND | — |
| **11** | α |  | 9.22 | 16.0 | 1.7 | 8.91 | >50 | 30.98 | 3.5 |
| **12** | α |  | 7.16 | 12.6 | 1.8 | 6.55 | >50 | 27.18 | 4.1 |
| **13** | α |  | 5.59 | 7.3 | 1.3 | 5.76 | >50 | 27.56 | 4.8 |
| Artemisinin | | | 25.5 | 25.4 | 1.0 | ND | | | |
| DHA | α:β (1:1) | | 4.1 | 3.0 | 0.75 | ND | | | |
| Chloroquine | | | 30.6 | 145.4 | 4.7 | 9.67 | | | |
| Cisplatin | | | ND | ND | - | 0.142 | > 100 | 21.686 | - |

ND, not determined.
[a] RI, resistance index, calculated as [$IC_{50}$ (Dd2)]/[$IC_{50}$ (D10)].

as solvent as it was capable of dissolving the extremely polar quinoline amines (a solvent that is unable to fully dissolve the quinoline amine would create an environment where there was more DHA acrylate than quinoline amine in the solution phase, which would in turn encourage acrylate polymerization). The compounds **4a**–**d** and **5** were synthesized using the DHA acrylate intermediate **1β**, and hence all bore the β-stereochemistry at C-10. The effect of the C10 orientation was envisaged to be studied by comparing the activities of acrylates **1α** and **1β**. Purification by silica gel column chromatography yielded the target compounds in low to moderate yields.

Apart from the target compounds where 7-chloro-4-aminoquinoline amines were added to the DHA acrylate, commercially available amines were also used in the conjugate addition (Scheme 4). Acetonitrile was used as the solvent, as there were no solubility problems with these low-molecular weight amines. After the necessary reaction time, the solvent was simply removed under reduced pressure, and the crude material purified by silica gel column chromatography to furnish the final compounds **6**–**13** in moderate to good yields. These additional compounds were synthesized using the DHA acrylate intermediate **1α**, and hence all bore the α-stereochemistry at C-10.

Antiplasmodial results against D10 and Dd2 strains of *P. falciparum* (Table 1) showed that the target compounds **4**-**13** as well as the DHA acrylate intermediates **1α** and **1β** displayed intermediate activities relative to DHA and chloroquine, that is, more active than chloroquine but less active to varying degrees than DHA in Dd2. In D10, a number of derivatives (**7**, **9** and **13**) showed comparable activity to DHA.

Also apparent was that the α- and β-acrylates (**1α** and **1β**) synthesized displayed comparable activities, suggesting that the orientation at C-10 does not significantly influence the in vitro activity of this type of compounds. This is unsurprising as both **1α** and **1β** show comparable activity; also, (β)-artesunate and (α)-artelinic acid have different orientations at C-10, but are both active antimalarials.[29]

The activities of the quinoline amine precursors **2a**–**c** against the CQR Dd2 strain were much lower than against the CQS D10 strain (as indicated by the high resistance indices, RI, of >4.0), a manifestation of cross-resistance with chloroquine. The activities of intermediates **2a**–**d** and **3** were also several times lower than their corresponding DHA derivatives **4a**–**d** and **5**. The cross-resistance noted above with some of the quinoline intermediates was absent in these derivatives (RI values typically around 1), showing that the incorporation of DHA aids 4-aminoquinolines in circumventing cross-resistance. Additionally, a general decrease in in vitro activities with increasing length of side-chains was observed for the target derivatives **4a**–**d**.

Interestingly, the conjugate adducts with commercially available amines were also quite active, and even exhibited better activities than those containing quinoline moieties. The most active compounds out of this series were **7**, **9** and **13**, all exhibiting $IC_{50}$ values of <10 nM against both strains of the malaria parasite. This may indicate that the DHA component is generally responsible for the majority of the antimalarial activity exhibited by all the conjugate adducts studied, and that the amine components of **6**–**13** (and similarly the quinoline-amine components of **4a**–**d** and **5**) simply serve to enhance this activity to varying extents.

Cytotoxic activities of these compounds were assessed in HeLa cells (human adenocarcinoma of the cervix cells), using cisplatin as the control (Table 1). Compound **4d** showed remarkable cytotoxicity against the HeLa cell line, with an $IC_{50}$ of 0.37 μM; the other DHA conjugates displayed moderate cytotoxic activities, with $IC_{50}$ values in the low micromolar range. These results prompted further tests against non-cancerous cells. Selected compounds (based on cytotoxic activities in HeLa cells) were tested against primary resting and phytohaemagglutinin (PHA)-stimulated lymphocytes. Primary resting lymphocytes occur naturally in the body, undergoing normal cell cycles. When these cells are stimulated by PHA, the speed of their cell cycles is increased, mimicking that of cancerous cells without being overtly cancerous themselves. These therefore represent normal, primary cell lines commonly used as controls for cancer cell lines. The activities of the selected compounds are also tabulated in Table 1.

The selected compounds were found to be relatively less toxic to the non-cancerous lymphocyte cell-lines, though they exhibited low tumor specificity (calculated by taking the average of the $IC_{50}$ in resting and PHA-stimulated primary lymphocytes and dividing it by the $IC_{50}$ in HeLa cells). Only one compound, **4d**, displayed good potential, with a tumor specificity of 31.

In summary, compounds, **7**, **9** and **13** displayed the most potent activities against D10 and Dd2 strains of *P. falciparum*. Their low toxicity to primary human cell lines suggested a potentially large therapeutic index for these compounds. In terms of development as possible anticancer agents, **4d** exhibited significant tumor specificity, and was identified as a good potential lead.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.090.

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