ARTICLE II

Artemisinin–Quinoline Hybrid-Dimers: Synthesis and *In Vitro* Antiplasmodial Activity

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Artemisinin–quinoline hybrid-dimers: Synthesis and in vitro antiplasmodial activity

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ABSTRACT

Novel artemisinin–quinoline hybrid-dimers were synthesized from dihydroartemisinin and different aminoquinolines at elevated temperatures (90–110 °C). All compounds were obtained as the β-isomers and were tested against both chloroquine sensitive and resistant strains of *Plasmodium falciparum*. Hybrid-dimer **8** showed the highest antiplasmodial activity, inheriting the optimum chain length of three carbon atoms.

Dimers containing a 1,2,4-trioxane unit exhibit potent in vitro antimalarial, antiproliferative, antitumor and anticancer activities.[1–4] Posner et al. synthesized C-10 non-acetal dimers by joining two 10-deoxoartemisinin trioxane units via a *para*-diacetylbenzene linker, resulting in ketone dimers with 2–5 times higher potency than artemisinin with highly selective and powerful anticancer activities.[5] Slade and et al.'s dihydroartemisinin (DHA) (**1**) acetal dimers, with diverse functionalized linker units, were appreciably more active than artemisinin and displayed enhanced anticancer activity.[6]

Hybrid molecules developed into an emerging strategy within medicinal chemistry and drug discovery and offer a simpler and more effective way to deliver these agents.[7] When a trioxane moiety was linked to an aminoquinoline entity, forming a dual molecule, it showed efficient antimalarial activity without recrudescence.[8–11]

The quinoline moiety serves as the pharmacophore for all the classic antimalarial drugs. In this study two artemisinin moieties were linked to different aminoquinolines resulting in a hybrid-dimer molecule. These disubstituted products were formed in low yields at elevated temperatures.

DHA was supplied as a mixture of epimers, but only the β-isomer was obtained during the first step of the synthetic procedure as was confirmed by X-ray crystallography.[12] Therefore, all synthesized dimers were obtained as the β-isomers and tested as such.

DHA was reacted with bromoethanol in the presence of boron trifluoride etherate and yielded 2-(10β-dihydroartemisinoxy) ethylbromide (**2**)[13] which was treated with different aminoquinoline moieties **3–6**, under specific conditions for the disubstitution to take place, forming novel hybrid-dimers **7–10**[14] (see Scheme 1).

The second DHA-moiety bound to the most available N-atom. The N-atom on C-4 is made unavailable due to resonance in the quinoline ring. Therefore, contrary to the other compounds, a quaternary ammonium salt formed with hybrid-dimer **8**.[15,16] The aminoquinolines were obtained according to previously reported procedures.[17–19]

In vitro antiplasmodial activity was determined against the chloroquine sensitive D10 strain and chloroquine resistant Dd2 strain of *Plasmodium falciparum* using a well established method.[20–22] In vitro cytotoxicity was conducted against a mammalian cell-line, Chinese Hamster Ovarian (CHO) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay.[20,23,24]

Compounds **7** and **8** inherited excellent antimalarial activity against the CQS and CQR strain, showing greater activity than CQ against both strains of the parasite. Moderate activity was displayed by compound **10**. Hybrid-dimer **9** was the least promising compound; although its RI were the lowest indicating good selectivity towards the CQR strain. However, all compounds were less active than DHA. All compounds showed good selectivity towards *P. falciparum* (SI ⩾ 60) (see Table 1). In this series a chain length of three carbon atoms, as in hybrid-dimer **8**, was found to be the optimum for antiplasmodial activity.

Yields could be increased if disubstitution is the primary focus, as these hybrid-dimers formed as byproducts. Good antiplasmodial

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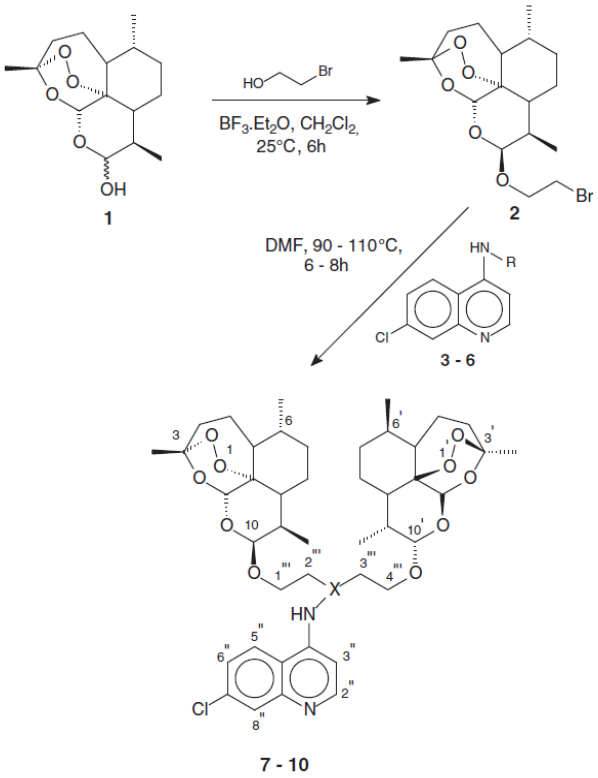
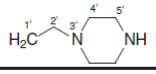
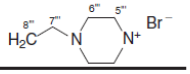


	R		X
3	$(CH_2)_2NH_2$	7	$(CH_2)_2N$
4	$(CH_2)_3NH_2$	8	$(CH_2)_3N$
5	$(CH_2)_4NH_2$	9	$(CH_2)_4N$
6		10	

Scheme 1. Synthesis of artemisinin–quinoline hybrid-dimers (**7–10**).

Table 1
In vitro IC_{50} values with standard deviations (SD) of antiplasmodial activity and cytotoxicity

Compound	D10: IC_{50} (nM)	SD	Dd2: IC_{50} (nM)	SD	RI	CHO: IC_{50} (10^3 nM)	SD	SI
7	7.37	0.47	46.08	0.57	6	10.00	6.77	1357
8	5.31	0.67	28.43	1.17	5	0.68	0.65	128
9	89.00	5.71	205.42	10.07	2	5.43	0.62	61
10	19.62	1.76	55.68	15.20	3	74.82	18.06	3813
CQ (n = 3)	21.54	6.73	157.90	52.70	7	ND	ND	ND
DHA (n = 4)	5.11	0.64	2.09 (n = 1)	0.33	0.4	ND	ND	ND
Emetine (n = 3)	ND	ND	ND	ND	ND	0.19	0.05	ND

Resistance index (RI) = IC_{50} Dd2/IC_{50} D10. Selectivity index (SI) = IC_{50} CHO/IC_{50} D10. ND = not determined. CHO = Chinese Hamster Ovarian.

activity and selectivity was inherited by the monosubstituted compounds.[18] As the in vitro testing was only used for screening, in vivo testing will give a better view in terms of activity, half-life and kinetics especially when compared to that of DHA. And thereby the concept of these novel hybrid-dimer compounds could serve as a foundation for further investigation.

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14. *General procedure for the synthesis of hybrid-dimers* **9**–**12**. A method as described by Li et al. was used with an adjustment of the temperature and reaction time. The temperature has to be kept between 90 and 110 °C and stirred for 6–8 h. MS spectra were included for compounds **9**–**12**. An isotopic pattern was observed with each compound and gave additional evidence of the presence of one chlorine atom in the radical cation. For NMR data only relevant information are given for compounds **10**–**12**. *1-(7-Chloro-4-(1,2-diaminoethyl)quinolyl)-2-(10β-dihydroartemisinoxy)ethyl dimer* **9**. Light yellow fluffy crystals. Mp: 88 °C R_f = 0.46 (DCM:MeOH 9:1). Yield: 15%. δ_H ($CDCl_3$) 8.48 (d, *J* = 5.3 Hz, 1*H*, H-2″), 7.92 (d, *J* = 2.0 Hz, 1*H*, H-8″), 7.64 (d, *J* = 8.9 Hz, 1*H*, H-5″), 7.37 (dd, *J* = 8.9, 2.1 Hz, 1*H*, H-6″), 6.32 (d, *J* = 5.4 Hz, 1*H*, H-3″), 5.98 (s, 1*H*, H-7‴), 5.29 (s, 2*H*, H-12; H-12′), 4.74 (d, *J* = 3.4 Hz, 2*H*, H-10; H-10′), 3.95 (dt, *J* = 10.8, 5.6 Hz, 2*H*, H-1α‴; H-4α‴), 3.55–3.50 (m, 2*H*, H-1β‴; H-4β‴), 3.27–3.18 (m, 2*H*, H-6‴), 3.05 (ddd, *J* = 12.6, 7.3, 5.2 Hz, 1*H*, H-5α‴), 2.95–2.89 (m, 1*H*, H-5β‴), 2.85–2.76 (m, 4*H*, H-2‴; H-3‴), 2.61–2.54 (m, 2*H*, H-9; H-9′), 2.29 (td, *J* = 14.1, 3.9 Hz, 2*H*, H-4α; H-4α′), 1.98–1.92 (m, 2*H*, H-4β, H-4β′), 1.38 (s, 6*H*, 3-Me, 3′-Me), 0.82 (d, *J* = 7.4 Hz, 6*H*, 9-Me; 9′-Me), 0.79 (d, *J* = 6.2 Hz, 6*H*, 6-Me; 6′-Me). δ_C ($CDCl_3$) 151.9 (C-2″), 149.6 (C-4″), 148.9 (C-8a″), 134.87 (C-7″), 128.6 (C-8″), 125.5 (C-6″), 121.2 (C-5″), 117.24 (C-4a″), 104.1 (C-3; C-3′), 102.4 (C-10; C-10′), 99.09 (C-3″), 87.8 (C-12; C-12′), 80.9 (C-12a; C-12a′), 67.6 (C-1‴; C-4‴), 53.9 (C-2‴; C-3‴), 52.9 (C-5‴), 52.3 (C-5a; C-5a′), 44.2 (C-8a; C-8a′), 39.9 (C-6‴), 37.3 (C-6; C-6′), 36.3 (C-4; C-4′), 34.4 (C-7; C-7′), 30.7 (C-9, C-9′), 26.1 (3-Me; 3′-Me), 24.5 (C-5; C-5′), 24.4 (C-8; C-8′), 20.2 (6-Me; 6′-Me), 13.0 (9-Me; 9′-Me). MS: *m*/*z*: 750 [$M^{\bullet+}$−2(CH_3O_2), 100%], 752 [$M^{\bullet+}$+2−2(CH_3O_2), 30%]; 796 [$M^{\bullet+}$−CH_3O_2]; 842 [$M^{\bullet+}$]. *1-(7-Chloro-4-(1,3-diaminopropyl)quinolyl)-2-(10β-dihydroartemisinoxy)ethyl dimer* **10**. Light yellow fluffy crystals. Mp: 84 °C. R_f = 0.73 (DCM:MeOH 9:1). Yield: 20%. δ_H ($CDCl_3$) 7.18 (s, 1*H*, H-8‴), 5.28 (s, 2*H*, H-12; H-12′), 4.71 (d, *J* = 3.4 Hz, 2*H*, H-10; H-10′), 3.94 (dt, *J* = 10.9, 5.6 Hz, 2*H*, H-1α‴; H-4α‴), 3.56–3.50 (m, 2*H*, H–H-1β‴; H-4β‴), 3.35 (dd, *J* = 10.2, 5.7 Hz, 2*H*, H-7‴), 2.84–2.79 (m, 4*H*, H-2‴; H-3‴), 2.77 (dd, *J* = 11.6, 5.8 Hz, 1*H*, H-5α‴), 2.74–2.69 (m, 1*H*, H-5β‴), 1.91–1.85 (m, 2*H*, H-6‴), 1.36 (s, 6*H*, 3-Me; 3′-Me), 0.84–0.80 (m, 12*H*, 9-Me; 9′-Me; 6-Me; 6′-Me). δ_C ($CDCl_3$) 66.7 (C-1‴; C-4‴), 54.3 (C-2‴; C-3‴), 54.1 (C-5‴), 43.5 (C-7‴), 24.9 (C-6‴). MS: *m*/*z*: 764 [$M^{\bullet+}$−2(CH_3O_2), 100%], 766 [$M^{\bullet+}$+2−2(CH_3O_2), 30%]; 810 [$M^{\bullet+}$−CH_3O_2]; 856 [$M^{\bullet+}$]. *1-(7-Chloro-4-(1,4-diaminobutyl)quinolyl)-2-(10β-dihydroartemisinoxy)ethyl dimer* **11**. Brownish oil. R_f = 0.67 (DCM:MeOH 9:1). Yield: 12%. δ ($CDCl_3$) 5.62 (s, 1*H*, H-9‴), 5.37 (s, 2*H*, H-12; H-12′), 4.74 (d, *J* = 3.4 Hz, 2*H*, H-10; H-10′), 3.88 (dt, *J* = 10.4, 6.0 Hz, 2*H*, H-1α‴; H-4α‴), 3.44 (dt, *J* = 10.4, 6.1 Hz, 2*H*, H-1β‴; H-4β‴), 3.29 (dd, *J* = 11.9, 5.7 Hz, 2*H*, H-8‴), 2.71 (dtd, *J* = 19.8, 13.5, 6.1 Hz, 4*H*, H-2‴, H-3‴), 1.79–1.74 (m, 2*H*, H-7‴), 1.39 (s, 6*H*, 3-Me; 3′-Me), 0.89 (d, *J* = 6.2 Hz, 6*H*, 9-Me; 9′-Me), 0.85 (d, *J* = 7.4 Hz, 6*H*, 6-Me; 6′-Me). δ_C ($CDCl_3$) 66.8 (C-1‴; C-4‴), 54.3 (C-2‴), 54.2 (C-3‴), 26.3 (C-7‴), 25.7 (C-8‴). MS: *m*/*z*: 778 [$M^{\bullet+}$−2(CH_3O_2), 100%], 780 [$M^{\bullet+}$+2−2(CH_3O_2), 30%]; 824 [$M^{\bullet+}$−CH_3O_2]; 870 [$M^{\bullet+}$]. *1-(7-Chloro-4-(2-piperazinyl-ethylamino)quinolyl)-2-(10β-dihydroartemisinoxy)ethyl dimer* **12**. Brown-yellow oil. R_f = 0.57 (DCM:MeOH 9:1). Yield: 18%. δ_H ($CDCl_3$) 5.35 (s, 2*H*, H-12; H-12′), 4.73 (d, *J* = 3.3 Hz, 2*H*, H-10; H-10′), 3.85 (dt, *J* = 10.5, 6.0 Hz, 2*H*, H-1α‴; H-4α‴), 3.44–3.41 (m, 2*H*, H-1β‴; H-4β‴), 3.16 (s, 4*H*, H-6‴), 2.78–2.61 (m, 10*H*, H-2‴; H-3‴; H5‴; H-8‴), 2.50 (t, *J* = 6.2 Hz, 2*H*, H-7‴), 1.36 (s, *J* = 7.1 Hz, 6*H*, 3-Me; 3′-Me), 0.88 (d, *J* = 6.3 Hz, 6*H*, 9-Me; 9′-Me), 0.84 (d, *J* = 7.4 Hz, 6*H*, 6-Me; 6′-Me). δ_C ($CDCl_3$) 66.9 (C-1‴;C-4‴), 57.0 (C-7‴), 54.5 (C-5‴), 53.4 (C-2‴; C-3‴), 52.4 (C-8‴), 52.0 (C-6‴). MS: *m*/*z*: 819 [$M^{\bullet+}$−2(CH_3O_2), 100%], 821 [$M^{\bullet+}$+2−2(CH_3O_2), 30%]; 865 [$M^{\bullet+}$−CH_3O_2]; 911 [$M^{\bullet+}$].
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