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Kojic acid derived hydroxypyridinone–chloroquine hybrids: Synthesis, crystal structure, antiplasmodial activity and β-haematin inhibition

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ABSTRACT

Aminochloroquinoline–kojic acid hybrids were synthesized and evaluated for β-haematin inhibition and antiplasmodial activity against drug resistant (K1) and sensitive (3D7) strains of *Plasmodium falciparum*. Compound **7j** was the most potent compound in both strains (IC_{50}^{3D7} = 0.004 μM; IC_{50}^{K1} = 0.03 μM) and had the best β-haematin inhibition activity (0.07 IC_{50} equiv vs 1.91 IC_{50} equiv for chloroquine). One compound **8c** was found to be equipotent in both strains (IC_{50} = 0.04 μM).

Malaria is a lethal human parasitic disease with over 200 million annual cases reported worldwide in 2010.[1] The World Health Organization estimated 660,000 deaths due to malaria worldwide of which 90% were in the African region and the remainder in the South East Asia and eastern Mediterranean regions.[1]

The problem of endemic malaria has been exacerbated by the emergence of widespread resistance to the once effective first line treatment drugs such as chloroquine (CQ) and sulphadoxine-pyrimethamine,[2] and more recently early indications of resistance to artemisinin monotherapy.[3,4] Artemisinin based combination therapies, ACTs, have replaced the failed therapies and are the recommended first line treatments of *falciparum* malaria in all endemic countries. With increasing resistance to other available agents, intensive drug discovery efforts aimed at developing new antimalarial drugs or modifying existing ones are on-going.[5]

It has been reported that an elevated host iron level is a serious risk factor for human malaria.[6] This has been observed in pregnant women in the 3rd trimester of gestation and in non-pregnant persons on iron supplements.[6–9] Antimalarial drugs that can address the excess physiologic iron load problem may be beneficial to infected pregnant mothers and persons with excess physiologic iron.

Thus antiplasmodial iron chelators have such a potential.

Iron chelators especially 3,4-hydroxypyridinones (3,4-HPOs) have been proposed as potential antimalarial agents because of the central role of iron for the rapid proliferation of the malaria parasite and the arrest of parasite growth by iron chelators in vivo and in vitro. An example is deferiprone, an orally bioavailable 3,4-HPO that is used for the treatment of iron overload or β-thallasaemia.[10–14] On the other hand 4-aminoquinoline based compounds continue to be utilized as starting parts for new antimalarial agents capable of circumventing aminoquinoline drug resistance.[2]

The rationale for pharmacophore hybridization of various bioactive agents is to increase their therapeutic potential.[15–17] This may be achieved through improvement in treatment efficacy in part through improvement in the pharmacokinetic profile of individual moieties through the resulting single molecular entity, reduction in the emergence and spread of drug resistance due to structural novelty of the hybrid as well as decrease in dose-dependent toxicity as the hybrid may be used at lower doses due to improved efficacy over individual components. In addition to these aforementioned potential benefits, hybridization provides synthetic advantages as it leads to structural novelty and diversity.[17]

Recently we demonstrated that the incorporation of the maltol-derived 3,4-HPO moiety into aminochloroquinolines by molecular

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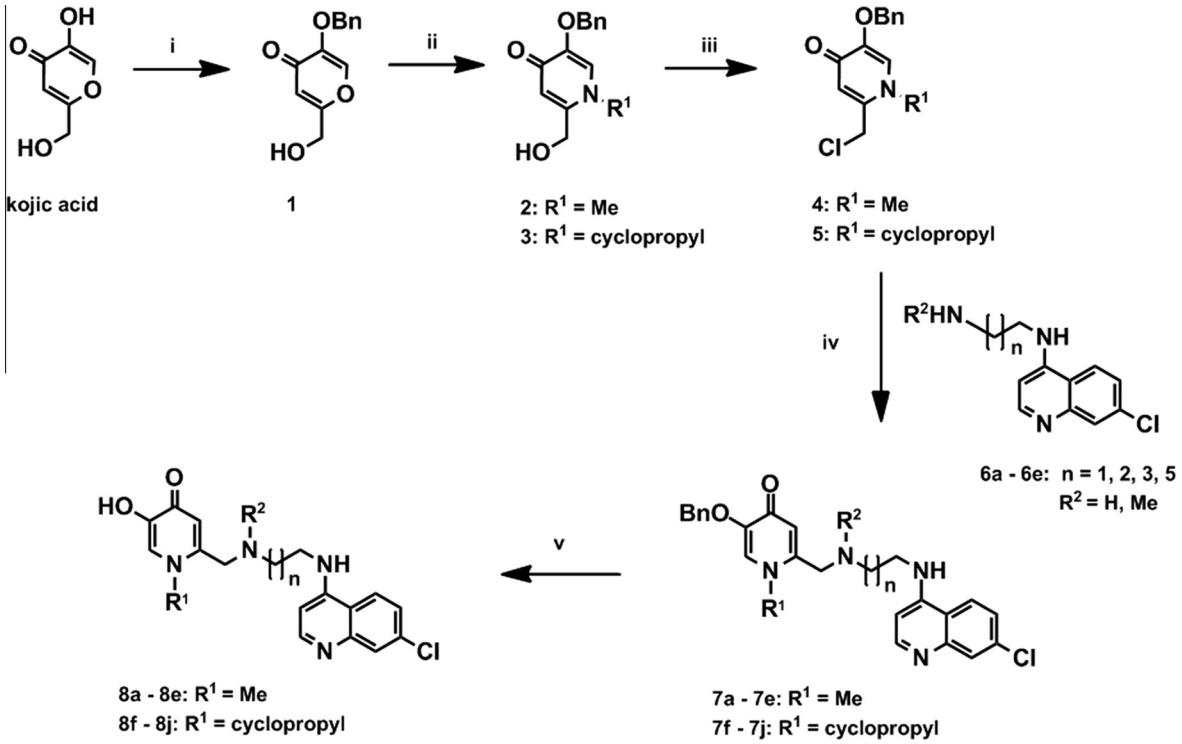
hybridization led to enhanced activity against drug resistant *Plasmodium falciparum*.[18] In a bid to optimize the antiplasmodial activity of these hybrid compounds, basic amino groups were introduced in the side chain adjacent to the 3,4-HPO moiety. This modification was intended to enhance accumulation of the drug in the parasitic food vacuole, lysosomes and other acidic intracellular vacuoles.[19,20]

The antiplasmodial effect of structural modification of these 3,4-HPO-chloroquine hybrids by incorporation of a basic amino group in the side chain is further explored in this communication using kojic acid. Herein we report the synthesis and mechanistic investigation of kojic acid-derived 3,4-HPO-chloroquine hybrid compounds and investigation of their mode of antiplasmodial activity with respect to inhibition of haemozoin formation.[21] Kojic acid was chosen as a precursor to the 3,4-HPO scaffold due to its synthetic accessibility and ease of synthetic manipulation.[22]

The Synthesis of the target compounds **1**, **2**, **3**, **4**, **5** and *N*-(7-chloro-4-quinolinyl)diaminoalkanes **6a**–**6e**, as illustrated in Scheme 1 is based on documented information with some modifications.[19,23–26] Chemoselective O-benzylation of the 5-hydroxyl group in the presence of the 2-hydroxymethyl substituent was observed. The Michael addition of methylamine or cyclopropylamine gave the pyridinone **2** or **3**. Chlorination of the pyridinones using neat thionyl chloride afforded the alkylhalide intermediates **4** or **5**. These intermediates were easily isolated as precipitates or crystals in good yields. The final step to the benzyl protected hybrids involved reaction of **4** or **5** with the appropriate *N*-(7-chloro-4-quinolinyl) diaminoalkane (**6a**–**6e**). The nucleophilic substitution reaction was successful under both microwave and reflux conditions. A combination of $NaHCO_3$ and triethylamine were used as bases to optimize the neutralization of the HCl byproduct. Purification of the benzylated conjugates was achieved by a combination of column chromatography, crystallization and preparative HPLC. Deprotection was achieved with palladium-catalysed hydrogenolysis or by acid-catalysed hydrolysis. The target compounds (**7a**–**7j** and **8a**–**8j**) were characterised by NMR, HR-MS, elemental analysis and mp. The elemental analyses indicated the molecules to be hydrated and this was further corroborated by the proton NMR spectra and the X-ray crystallographic data of one of the compounds; **7c** (Figs. 1 and 2).

The X-Ray crystallographic analysis of **7c** indicated that one methanol and three water molecules were incorporated for each molecule of **7c** ($M{\cdot}3H_2O{\cdot}CH_3OH$). The methanol molecule is disordered by alternating of the positions of C and O, which were refined isotropically due to their large thermal motions. Two of the water molecules are disordered with oxygen over two positions. The hydrogen atoms on these disordered methanol and water molecules were excluded from the final structure model. For the main molecule, all non-hydrogen atoms were refined anisotropically and all hydrogen atoms on carbons were positioned geometrically with C–H distances ranging from 0.95 Å to 1.00 Å and refined as riding on their parent atoms, with U_{iso} (H) = 1.2–1.5 U_{eq} (C). The position of amine hydrogen H_2N (on N-2) was located in the difference electron density maps and refined with simple bond length constraints. The structure was refined successfully with a R factor of 0.0579.

The parameters for crystal data collection and structure refinements are presented in the supplementary data. The data was also deposited at the the Cambridge Crystallographic Data Centre. The deposit number is CCDC 1006985. Further analysis showed that the heterocyclic ring is not regular as it has two C–C [1.436 (3), 1.419(3)], two C=C [1.364(3), 1.343(2)] and two C–N [1.371(3), 1.353(3)], bonds, respectively. The C(17)–O(1) bond (1.276 Å) is significantly longer than a pure ketone C=O bond (1.210 Å),[25] this provides O(1) with a partial negative charge that is used to form strong hydrogen bonds. The C(17)–O(1) bond length is similar to



Scheme 1. (i) BnCl (1.1 equiv), NaOH (1.1 equiv), EtOH, reflux, 24 h; (ii) CH_3NH_2 or cyclopropylamine (1.8 equiv), NaOH (pH >10), 50% aq EtOH, reflux, 12 h; (iii) $SOCl_2$ (8 equiv), −7 °C; stir, 2–12 h, to ambient temperature; (iv) *N*-(7-chloro-4-quinolinyl) diaminoalkane (1.2 equiv), DMF, Et_3N (1.2 equiv), Na_2CO_3 (1.2 equiv), reflux 2-24 h or *N*-(7-chloro-4-quinolinyl) diaminoalkane (1 equiv), AcN, NaOH (s) (1.1 equiv), microwave, 18 min., 100 °C, 250W, 249 bars; (v) 2 M ethanolic HCl, reflux 74 °C or H_2O/EtOH/concd HCl 1:2:3, H_2, Pd/C, 4 atm., 4–6 h. Note: structural details of the target compounds are in Table 2 and in the Supplementary information.

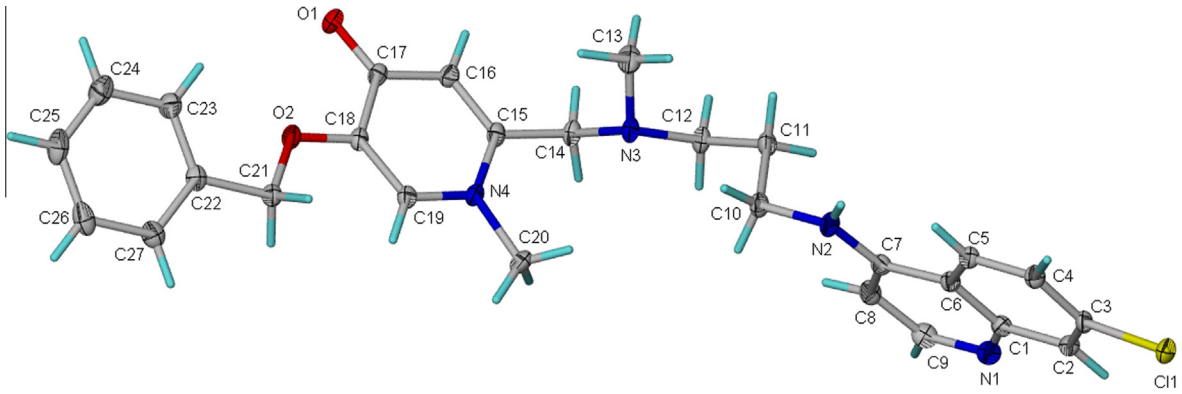


Figure 1. Crystal structure of **7c**.

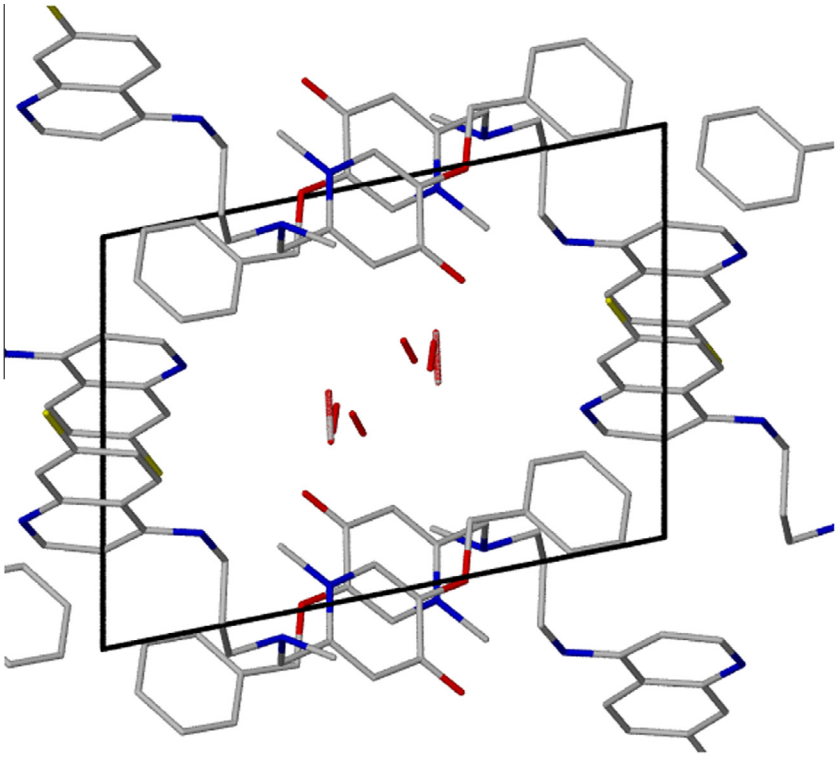


Figure 2. Projection viewed along b. Note the disordered solvent molecules (methanol and water) in the crystal of **7c**.

the corresponding bond length in the pyrone precursor, kojic acid (1.244 Å) and deferiprone (1.278 Å) a closely related pyridinone.[27,28] The ketone oxygen O(1) is not protonated because protonation would have resulted in the extension of the C(17)–O(1) bond length to ≈1.346 Å, the typical length of a protonated ketonic oxygen in a hydroxypyridinone.[25] Protonation is not expected since the compound was obtained as a free base and no acid was used in the recrystallization process. The water molecules are shown to participate in intramolecular hydrogen bonding along with the ketonic oxygen O(1) and the quinoline nitrogens N(1) and N(2) (Table 1).

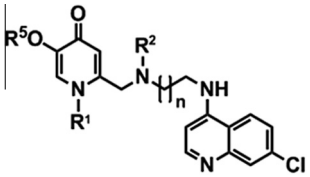
Table 1
Hydrogen bonds for **7c** [A and deg.]

D-H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N(2)–H(2N)...O(1)#1	0.956(10)	2.011(16)	2.908(2)	155(3)
O(1W)–H(1W1)...O(1)	0.966(10)	1.813(14)	2.767(3)	169(4)
O(1W)–H(1W2)...N(1)#2	0.961(10)	1.779(11)	2.740(3)	178(4)

The most lipophilic compound **7j**, as determined from clogP,[29] exhibited the best β-haematin inhibition activity (Table 2). However, no trend or correlation was observed between β-haematin inhibition and lipophilicity in both deprotected and benzylated analogues. Generally, deprotection resulted in a decrease in β-haematin inhibition activity of these compounds except for **7a**. This observation implies the involvement of the benzyl group in the inhibition of β-haematin formation. The β-haematin inhibition activity of all compounds was superior or comparable to that of CQ (1.9 IC_{50} equiv) except for some deprotected analogues; **8c** (6.12 IC_{50} equiv), **8h** (5.24 IC_{50} equiv) **8g** (2.62 IC_{50} equiv) and **8i** (2.57 IC_{50} equiv).

The introduction of the tertiary amino group resulted in a decrease in β-haematin inhibition activity in both the benzylated and deprotected analogues, for example, **7b** (IC_{50} equiv = 1.3) compared to **7c** (IC_{50} equiv = 1.98); **7g** (IC_{50} equiv = 0.22) compared to **7h** (IC_{50} equiv = 1.87); **8g** (IC_{50} equiv = 2.62) compared to **8h** (IC_{50} equiv = 5.24). The only exception was the deprotected analogue, **8b** (6.12 IC_{50} equiv) when compared to **8c** (1.95 IC_{50} equiv).

Table 2
In vitro antiplasmodial and β-haematin inhibition activities of target compounds[a]



Compd	*n*	R^1	R^2	R^5	*c*LogP	βHIA[b] IC_{50} (equiv)	3D7 IC_{50} (μM)	K1 IC_{50} (μM)	RI[c]
7a	1	Me	H	Bn	2.7	1.27	0.54	2.56	4.7
8a	1	Me	H	H	1.0	0.63	0.58	1.75	3.0
7b	2	Me	H	Bn	3.2	1.30	0.30	2.08	6.9
8b	2	Me	H	H	1.5	6.12	0.12	0.27	2.2
7c	2	Me	Me	Bn	3.7	1.98	0.39	0.15	0.4
8c	2	Me	Me	H	2.1	1.95	0.04	0.04	1.0
7d	3	Me	H	Bn	3.7	0.42	0.24	1.71	7.1
8d	3	Me	H	H	2.0	ND	0.67	0.07	0.1
7e	5	Me	H	Bn	4.7	0.51	0.04	0.32	8
8e	5	Me	H	H	3.0	0.70	0.19	0.32	1.7
7f	1	Cyclopropyl	H	Bn	3.4	0.56	0.21	1.45	7.2
8f	1	Cyclopropyl	H	H	1.6	1.41	0.23	0.36	1.6
7g	2	Cyclopropyl	H	Bn	3.9	0.22	0.19	0.27	1.4
8g	2	Cyclopropyl	H	H	2.0	2.62	0.20	0.72	3.6
7h	2	Cyclopropyl	Me	Bn	4.4	1.87	0.15	0.97	6.5
8h	2	Cyclopropyl	Me	H	2.6	5.24	0.30	0.46	1.5
7i	3	Cyclopropyl	H	Bn	4.3	0.82	0.16	0.85	5.3
8i	3	Cyclopropyl	H	H	2.6	2.57	0.17	0.36	2.1
7j	5	Cyclopropyl	H	Bn	5.3	0.071	0.004	0.03	7.5
8j	5	Cyclopropyl	H	H	3.6	1.39	0.03	0.08	2.7
ART							0.009	0.004	0.4
CQ					5.3	1.9	0.016	0.20	4.7

[a] SE ⩽ 7%, $n = 3$.
[b] βHIA, β-haematin inhibition activity.
[c] RI, resistance index calculated as [IC_{50}(K1)/IC_{50}(3D7)], ND, not determined. *c*Log*P*, calculated partition coefficient.

The β-haematin inhibition activity was generally observed to improve on replacement of the pyridinone *N*-methyl with a *N*-cyclopropyl group with one exceptional pair; **7i** and **7d**.

Antiplasmodial activity against the 3D7 strain was observed to increase with increase in lipophilicity. Only compound **7j** (IC_{50} = 0.004 μM) was more active than chloroquine (IC_{50} = 0.016 μM) against the sensitive strain 3D7. A decrease or an increase in antiplasmodial activity against the 3D7 strain upon deprotection was found to be compound specific.

The decreased β-haematin inhibition did not translate to lower antiplasmodial activity in all the deprotected analogues. For instance significant differences in β-haematin inhibition activities of compounds with the cyclopropyl group were observed but their antiplasmodial activities in the 3D7 strain appeared to be similar for most of them (except for compounds **7j** and **8j**). This may imply that inhibition of haemozoin formation may not be the only mode of action responsible for their antiplasmodial activity or that differences in cellular uptake are involved.

Among the benzylated analogues, antiplasmodial activity in the resistant K1 strain was observed to increase with increase in lipophilicity (length of alkyl side chain). However, no trend could be discerned for the deprotected analogues. The antiplasmodial activity was found to improve on deprotection for most compounds implying susceptibility of the resistant strain to, presumably, the hydroxypyridinone moiety. This is further corroborated by the low resistance indices observed for deprotected analogues when compared to the benzylated ones (Table 2). In general many compounds showed cross resistance with chloroquine in being less active in the resistant strain than in the sensitive strain. However, five compounds, **8c**, **7c**, **7j**, **8d** and **8j** were more potent in the resistant strain than chloroquine.

In contrast to what was observed in the sensitive strain, the introduction of a tertiary amine group enhanced activity of the series in the resistant strain. This is clear when **7c** (IC_{50} = 0.15 μM) is compared to **7b** (IC_{50} = 2.08 μM) and **8c** (IC_{50} = 0.04 μM) compared to **8b** (IC_{50} = 0.12 μM).

Compound **8c** was identified as the only hybrid with a tertiary amine group that had its β-haematin inhibition potency correlate with to antiplasmodial potency in both the sensitive and resistant strain. All the *N*-methylpyridinones (except **8b**) were stronger β-haematin inhibitors than **8c** but the latter had superior antiplasmodial activity. Surprisingly the most potent β-haematin inhibitor **7d** among the *N*-methylpyridinones was not the most potent antiplasmodial. It may be assumed that factors, other than β-haematin inhibition are responsible for the antiplasmodial activity of **8c**. Presumably accumulation of this compound in the parasitic food vacuole is enhanced by the presence of the tertiary amino group.[30] Furthermore compounds **7c** and **8c** exhibited significantly higher antiplasmodial activity in the K1 strain than related analogues that lack the tertiary amine group (**7b** and **8b**).

All the deprotected compounds in the series had lower resistance indices (RIs) than chloroquine suggesting that they are likely to be active against resistant parasites. Generally the deprotected analogues exhibited lower RI values than their benzylated analogues and the introduction of the tertiary amine group improved the resistance indices of hybrid compounds with the *N*-cyclopropyl group.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.012. These data include MOL files and InChiKeys of the most important compounds described in this article.

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