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Synthesis and antiplasmodial activity of some 1-azabenzanthrone derivatives

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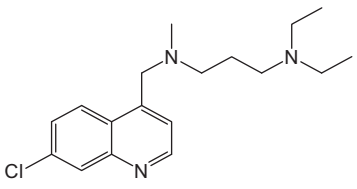
ABSTRACT

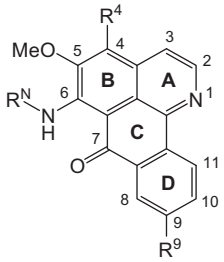
Some synthetic 1-azabenzanthrones (7*H*-dibenzo[*de*,*h*]quinolin-7-ones) are weakly to moderately cytotoxic, suggesting that they might also show antiparasitic activity. We have now tested a small collection of these compounds in vitro against a chloroquine-resistant *Plasmodium falciparum* strain, comparing their cytotoxicity against normal human fibroblasts. Our results indicate that 5-methoxy-1-azabenzanthrone and its 2,3-dihydro analogue have low micromolar antiplasmodial activities and showed more than 10-fold selectivity against the parasite, indicating that the dihydro compound, in particular, might serve as a lead compound for further development.

Malaria is the most prevalent insect–borne parasitic disease, caused by several protozoa belonging to the genus *Plasmodium* which are inoculated by *Anopheles* mosquitoes. Five *Plasmodium* species are responsible for malaria in humans: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. The first two are the most widespread, and *P. falciparum* the most deadly. Approximately 40% of the world's population is at risk, mainly in the poorest countries. Every year more than 500 million new cases of malaria arise, and more than one million people die of the disease.[1]

Control of malaria relies on control of the insect vectors and on chemotherapy using a variety of drugs. The first pure compound to be widely used against malaria was the quinoline alkaloid quinine. Subsequent variations on the quinine structure led to the development of a considerable number of synthetic aminoquinoline antimalarials, the most successful of which was chloroquine (**1**). Nevertheless, malaria therapy is plagued by the appearance of chloroquine-resistant *Plasmodium* strains, making the discovery of new drugs a priority.[2] One of the current approaches to this



Chloroquine (**1**)



(**2**) $R^N = CH_2CH_2Ph(4\text{-}OH)$; $R^4 = H$; $R^9 = OMe$
(**3**) $R^N = H$; $R^4 = R^9 = OMe$
(**4**) $R^N = R^4 = R^9 = H$

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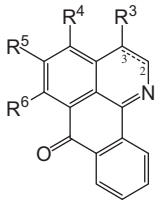
problem is the screening of plant extracts and secondary metabolites against the parasite.

The oxoisoaporphines, exemplified by structures **2**, **3** and **4**, constitute a small family of natural products characterised by their planar 7*H*-dibenzo[*de*,*h*]quinolin-7-one skeleton, commonly known in the dye and pigment industries as 1-azabenzanthrone.[3–5] Two oxoisoaporphines isolated from *Menispermum dauricum* DC, bearing a nitrogen substituent at C-6 of the B ring (daurioxoisoaporphines A and B, **2** and **3**), and lakshminine (**4**), isolated from *Sciadotenia toxifera* Krukoff & A.C. Smith, have demonstrated toxicity in several cell lines.[6] Due to their planar structure, these compounds could be expected to intercalate between DNA base pairs, and thus inhibit cell replication and possibly exhibit anticancer properties. They might also be cytotoxic as a consequence of their azaquinonoid structure, which could reasonably interfere with mitochondrial electron transport. While the literature records no data on the antimalarial activity of alkaloids of this family, a number of synthetic 1-azabenzanthrones have been screened and have been found to be weakly to moderately cytotoxic.[6,7] We have now tested a collection of synthetic 1-azabenzanthrones (including the oxoisoaporphine lakshminine—**4**) in vitro against a chloroquine-resistant *P. falciparum* strain.



4 R^3= H, R^4= H, R^5= MeO, R^6= NH_2; 2,3 ⟍
5 R^3= H, R^4= H, R^5= H, R^6= H; 2,3 ⟍
6 R^3= Br, R^4= H, R^5= H, R^6= H; 2,3 ⟍
7 R^3= Br, R^4= H, R^5= MeO, R^6= H; 2,3 ⟍
8 R^3= H, R^4= Br, R^5= MeO, R^6= H; 2,3 ⟍
9 R^3= H, R^4= H, R^5= MeO, R^6= H; 2,3 ⟍
9A R^3= H, R^4= H, R^5= MeO, R^6= H; 2,3 ⟋
10 R^3= H, R^4= H, R^5= MeO, R^6= MeO; 2,3 ⟍
11 R^3= H, R^4= H, R^5= MeO, R^6= OH; 2,3 ⟍
12 R^3= H, R^4= H, R^5= MeO, R^6= NO_2; 2,3 ⟍
13 R^3= H, R^4= NO_2, R^5= MeO, R^6= H; 2,3 ⟍
14 R^3= H, R^4= NH_2, R^5= MeO, R^6= H; 2,3 ⟍

1-Azabenzanthrone **5** was prepared improving on a published procedure (Scheme 1).[8] The synthesis of **5** was carried out in good yield and without side product formation. Aromatisation of **5A** (see below) by air oxidation over Pd/C similarly afforded **5**. The bromination of **5** in CH_3CN afforded **6** in poor yield.

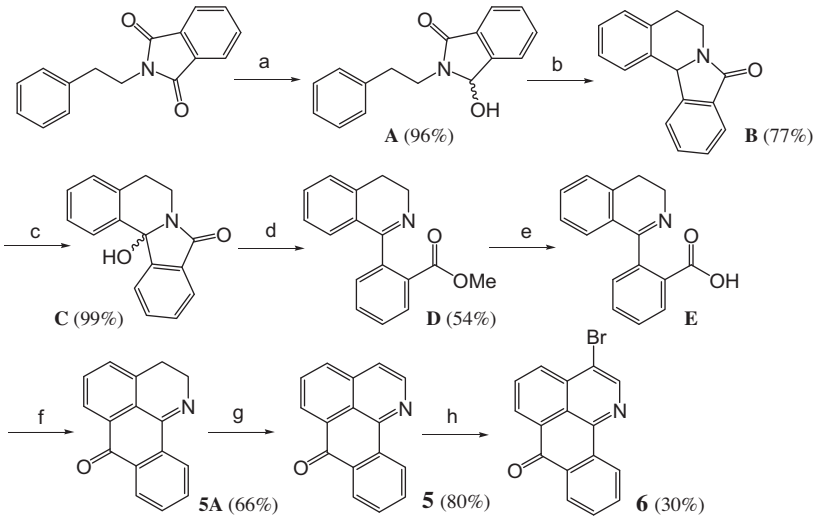
Although 1-azabenzanthrones **9**, **10** and **11** have been prepared previously by dehydrogenation of their 2,3-dihydro analogues, the low yields obtained following the published procedure of Walker and Kempton,[9] makes such an approach impractical for this purpose. Therefore, we have only used this route to obtain **9A**, **11A** and **10A** and have preferred the methodology used by Kunitomo et al.[10] as exemplified in Scheme 2 for the synthesis of **11**, which was subsequently methylated to afford **10**.

The bromination of **9** in CH_3CN afforded **7** and **8** in poor yields. The nitro (**12**, **13**) and amino (**4**, **14**) derivatives were prepared as published.[6]

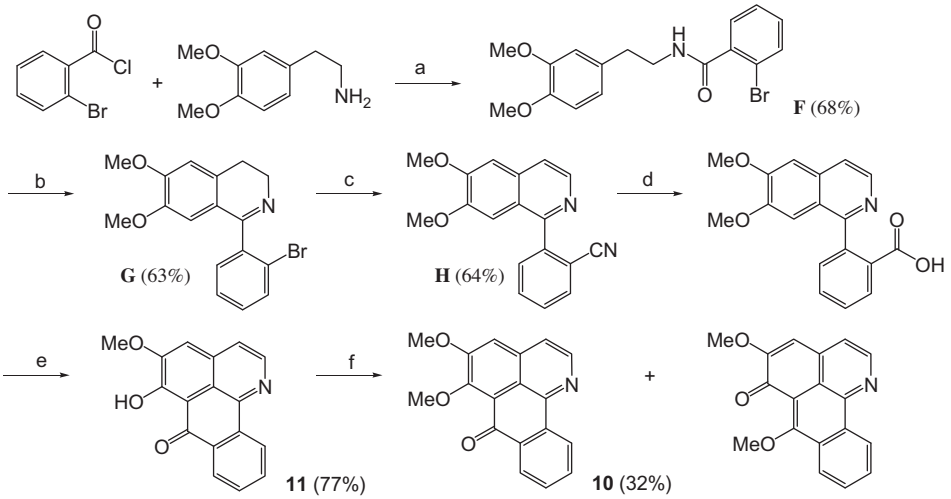
Cultures of the chloroquine-resistant FCR-3 strain of *P. falciparum* were grown at 37 °C in a 5% CO_2 atmosphere on RPMI 1640 medium supplemented with gentamycin 0.1 mg/mL and 10% heat-inactivated A^+ human serum, as previously described.[11] The synthetic derivatives, dissolved in DMSO, were added at final concentrations ranging from 250 to 0.1 μM. All experiments were repeated twice with two or three replicates each. The final DMSO concentration was never greater than 0.1%. In vitro antimalarial activity was measured using the [^{3}H]-hypoxanthine (MP Biomedicals, USA) incorporation assay.[12] Results were expressed as the concentration resulting in 50% inhibition (IC_{50}) that was calculated by a nonlinear regression logistic dose response model and the mean IC_{50} values and standard deviation for each compound was calculated. Values are means of n = 4–6.

The IC_{50} values of the tested 1-azabenzanthrones are collated in Table 1, together with their cytotoxicities to normal human fibroblasts and their selectivity indices (S.I.).[13]

Of the series of 1-azabenzanthrones tested, only the 5-methoxy compound (**9**) and its dihydro analogue (**9A**) exhibited low micromolar antiplasmodial activities. Although the latter is an order of magnitude less potent than the reference drug chloroquine (**1**), our results suggest that **9A** might serve as a lead compound for further development. Interestingly, when our values for **9** and **9A** are compared with the toxicities to normal human cells, these compounds showed more than 10-fold antiplasmodial selectivity while the others were either nonselective or more toxic to the fibroblasts than to the parasite. Although this series of compounds is insufficient to draw firm conclusions as to a structure–activity relationship, almost all the analogues with substituents on ring B have antiplasmodial activities at concentrations ⩽100 μM, while the unsubstituted 1-azabenzanthrone and its 3-bromo derivative have IC_{50} ⩾ 200 μM.



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH/dioxane, reflux, 3 h; (b) 37% HCl, reflux, 1 h; (c) 0.5 M KOH/MeOH, air, reflux, 24 h; (d) Me_2SO_4/MeOH, reflux, 3 h; (e) 37% HCl, reflux, 4 h; (f) H_2SO_4/SO_3, 0–5 °C, 24 h; (g) Pd/C, toluene, reflux, 24 h; (h) Br_2, CH_3CN, 80 °C, 24 h.



Scheme 2. Reagents and conditions: (a) Et_2O, 0–5 °C, 1 h; (b) $POCl_3$, toluene, reflux, 2.5 h; (c) CuCN, DMF, 180 °C, 6 h; (d) 40% KOH/EtOH, 100 °C, 48 h; (e) PPA, 100 °C, 1 h; (f) Ag_2O, MeI, MeOH/DCM, 60 °C, 6 h.

Table 1
In vitro activity of 1-azabenzanthrones vs *Plasmodium falciparum* and MRC-5 human lung fibroblasts

Compound	IC_{50} (μM) *P. falciparum*	IC_{50} (μM)[13] human fibroblasts	S.I.
1-Azabenzanthrone (**5**)	230	41.6	0.18
3-Bromo-1-azabenzanthrone (**6**)	260	>100	>0.38
3-Bromo-5-methoxy-1-azabenzanthrone (**7**)	220	28.9	0.13
4-Bromo-5-methoxy-1-azabenzanthrone (**8**)	52	>100	>1.99
5-Methoxy-1-azabenzanthrone (**9**)	6.4	>100	>15.6
5-Methoxy-2,3-dihydro-1-azabenzanthrone (**9A**)	2.5	81.3	32.4
5,6-Dimethoxy-1-azabenzanthrone (**10**)	74	50.5	0.68
5-Methoxy-6-hydroxy-1-azabenzanthrone (**11**)	51	65.1	1.27
5-Methoxy-6-nitro-1-azabenzanthrone (**12**)	56	80.0	1.42
5-Methoxy-6-amino-1-azabenzanthrone (**4**)	100	17.9	0.18
5-Methoxy-4-nitro-1-azabenzanthrone (**13**)	21	10.2	0.49
5-Methoxy-4-amino-1-azabenzanthrone (**14**)	100	37.0	0.37
Chloroquine (**1**)	0.19	N.T.	-

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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.2012.10.092.

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