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Antimalarial benzo[*c*]phenanthridines

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Abstract—Analogues of the antimalarial alkaloid nitidine have been prepared with high potency against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro. Simple modifications, using an established synthetic route, resulted in an analogue with IC_{50} below 5 ng/mL against a chloroquine-sensitive strain of *P. falciparum*. *N*-Ethylethoxidine had IC_{50} below 30 ng/mL against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*.

A bio-assay guided survey of some of the plants used by the Pokot tribe of Kenya, to treat fevers, identified two species with activity against *Plasmodium falciparum* in vitro.[1] Further work on one of the two species, *Toddalia asiatica*, showed that a major active component was the quaternary benzo[*c*]phenanthridine alkaloid nitidine **1**, with IC_{50} values against a range of chloroquine-sensitive and -resistant strains of *P. falciparum* in the range 42–165 ng/mL (0.1–0.4 μM). Nitidine showed no cross-resistance with chloroquine.

Fortuitously, several synthetic benzo[*c*]phenanthridines (BZPs) were available from previous work.[2,3,5] The same in vitro tests for antimalarial activity applied to the synthetic compounds showed that fagaronine **2** was relatively inactive, with IC_{50} in the range 0.8–1.0 μg/mL (Table 1). *O*-Methyl fagaronine **3**, however, was 6–30 times more potent than **2**. Values for **3** against the chloroquine-sensitive K39 strain of *P. falciparum* at ca. 30 ng/mL and the chloroquine-resistant V1/S strain at ca. 140 ng/mL (Table 1), were encouraging when compared with the values for chloroquine itself, at 4 and 57 ng/mL, respectively, given that chloroquine is one of the most potent antimalarial agents. Unlike nitidine **1**, *O*-methyl fagaronine **3** showed some degree of cross-resistance with chloroquine (Table 1). In contrast, the fully synthetic analogue **4** was equally active against both K39 and V1/S strains of *P. falciparum*, at 60 ng/mL, and this gave encouragement that analogue design could give compounds with both increased potency and lack of cross-resistance with chloroquine.

Table 1. In vitro activities (IC_{50}) of BZPs against two strains of *P. falciparum*

Compd.	K39 ng/mL (SD)	μM	V1/S ng/mL (SD)	μM	Ratio
2	1040 (250)	2.3	810 (70)	1.8	0.8
3	33 (10)	0.07	140 (42)	0.3	4.2
4	60 (4)	0.12	60 (20)	0.12	1.0
5	130 (30)	0.26	130 (20)	0.26	1.0
6	380 (70)	0.8	350 (60)	0.74	0.9
7	4.7 (4)	0.009	53 (6)	0.11	11.3
8	42 (8.6)	0.09	56 (20)	0.12	1.3
9	20 (9.9)	0.04	28 (12)	0.05	1.4
10	91 (44)	0.19	428 (58)	0.90	4.7
11	696 (191)	1.00	1288 (216)	1.84	1.9

The K39 strain is chloroquine sensitive and V1/S is resistant. In the same tests, chloroquine was active at 13 and 186 nM, respectively. The test method was exactly as described previously.[1] 'Ratio' is the MIC for the V1/S strain divided by that for the K39 strain.

The quaternary BZPs, particularly **2**, are effective inhibitors of human topoisomerases.[4,5] The relative lack of antimalarial activity of **2** (Table 1), suggests that the mode of action on malaria parasites differs from the

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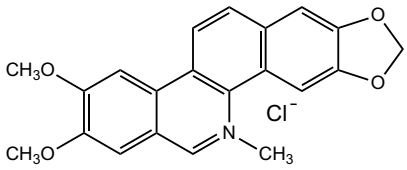
effects on human cancer cells, since **2** is one of the most effective BZPs against the human topoisomerases. Very recently, however, **2** has been reported to be very potent against a different strain of *P. falciparum* in vitro.[6]



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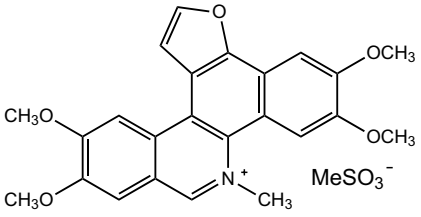

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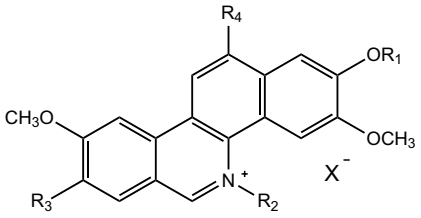
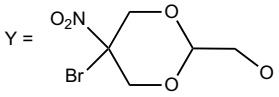


	R_1	R_2	R_3	R_4	X
2	H	Me	OMe	H	$MeSO_3^-$
3	Me	Me	OMe	H	$MeSO_3^-$
4	H	Me	OMe	OEt	$MeSO_3^-$
5	Me	Me	OMe	OEt	$MeSO_3^-$
6	Et	Me	OMe	H	$MeSO_3^-$
8	Me	Et	OMe	H	I^-
9	Me	Et	OMe	OEt	I^-
10	Me	Me	SMe	H	$MeSO_3^-$
11	Me	Me	OMe	Y	$MeSO_3^-$



The data for the previously synthesised BZPs **2**–**6** (Table 1) show that the activity is very sensitive to small changes in structure. While there is a gain in potency when OH at position 2 is changed to OMe (**2**–**3**), in both cases with R_4 = H, substitution of R_4 with OEt results in greater potency with OH at position 2, rather than OMe (**4** vs. **5**). Analogues **2**, **5** and **6** were relatively inactive, compared to **3** and **4**. These results encouraged the rational design of further analogues, **7**–**11**. The new compounds were characterised by a combination of NMR, FABMS, combustion analysis and HPLC.

Assuming that the BZPs are killing the parasites through an interaction with DNA, the most likely rationale is that they intercalate between the base pairs[7] and disrupt the shape of the double helix in its interactions with proteins; there is also the possibility of a covalent bond through C(6) of the BZP and an appropriately placed nucleophilic group of a topoisomerase in a tripartite complex.[8] It has been observed, however, that representative BZPs do not show isosbestic points when titrated against calf thymus DNA,[8] indicating that there may be more than one mode of binding. It is interesting that the biosynthetically related alkaloid berberine, always assumed to be an intercalator, has recently been shown to bind to the minor groove of DNA.[9] Berberine is also a potent antimalarial.[10]

Three modifications to structures **2**–**6** were proposed in the context of the possible mode of action. First, extension of the molecular area through cyclisation of a 12-*O*-acetal derivative (Scheme 1) to give **7** would increase the possibility of intercalation. In practice, **7** was the most potent analogue against chloroquine-sensitive *P. falciparum* (Table 1) with an IC_{50} less than 5 ng/mL, but showed some cross-resistance with chloroquine (Table 1).

Molecular modelling consistently shows that the quaternary BZPs are not flat. There is a steric interaction between the *N*-alkyl group and H(4), resulting in a substantial twist. It is possible that the twist matches the propeller twist of the DNA bases when intercalated, improving fit and affinity: propeller twist is generally greater in AT tracts[11] and the BZPs show increased affinity for such sequences.[7] Increasing the size of the *N*-substituent, from methyl to ethyl, gave analogues **8** and **9**.

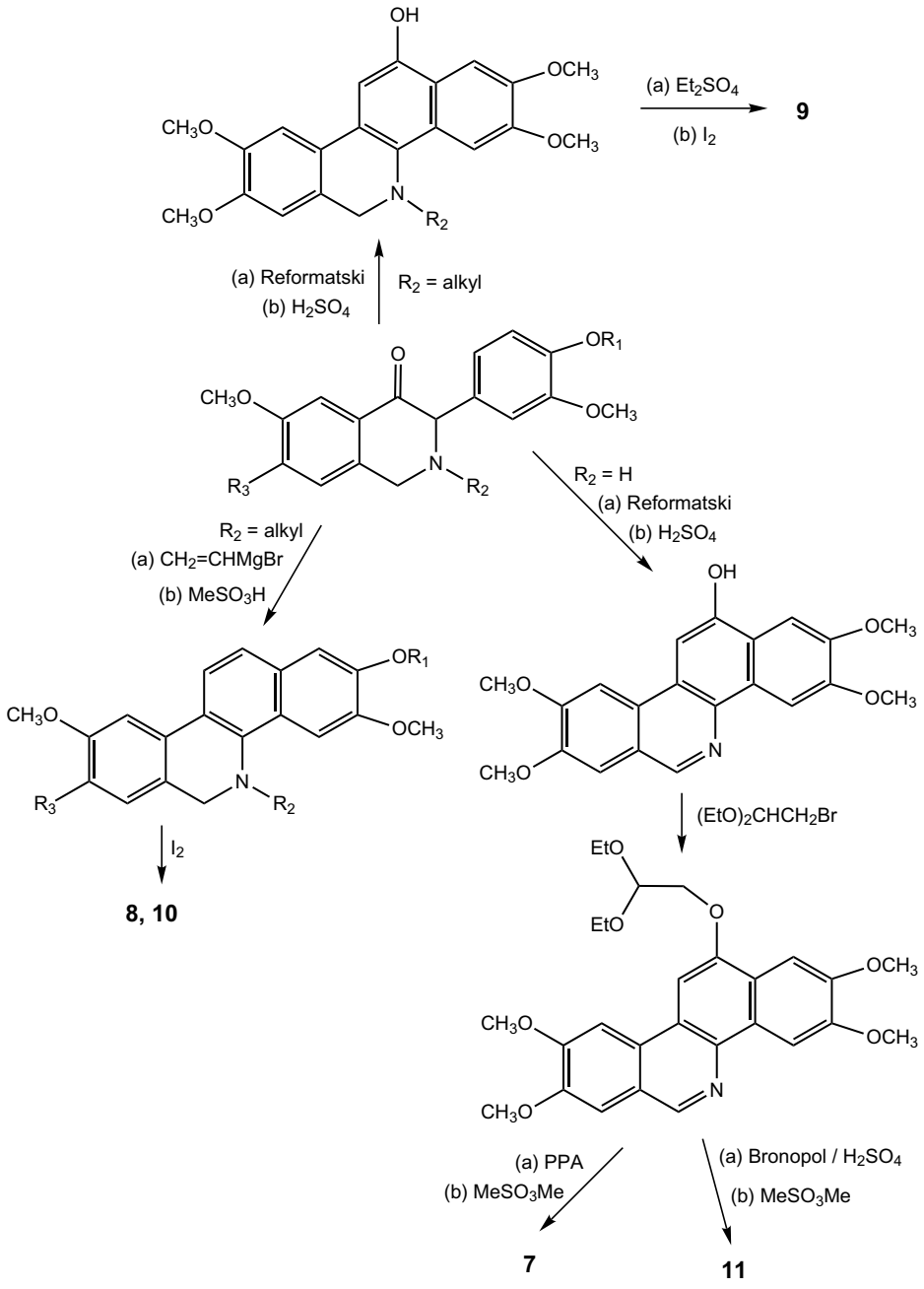
The analogue **8** with H as 12-substituent had good activity and little cross-resistance with chloroquine (IC_{50} 42 and 56 ng/mL), while 12-ethoxy substitution as in **9** increased the activity further (IC_{50} 20 and 28 ng/mL).

BZP analogues with *S*-methyl instead of *O*-methyl are potentially available by practical routes[12] and would be expected to show substantially increased lipophilicity. One such analogue (**10**) was prepared and showed decreased potency compared to the direct oxygen analogue **3**. There is no obvious explanation for this.

An attempt to increase potency by causing local DNA damage through incorporation of the free radical generator bronopol[13] gave **11**, the least active of the series (Table 1). Presumably the bronopol moiety is too bulky to permit DNA binding.

It is possible that, like berberine, the BZPs are binding in the minor groove of DNA as well as (or rather than) intercalating between the base pairs. Such an interaction would be likely to occur with positions 9, 10, 11, 12 on the inside of the curve, at the base of the groove, and might permit larger *N*-substituents to lie towards the outside of the helix, near the phosphate backbone. The inside of the BZP curve maps precisely onto the inside curve of typical minor groove binders such as netropsin (Fig. 1).

The existence of two modes of interaction might be connected with the observation that some of the present analogues show cross-resistance with chloroquine, while



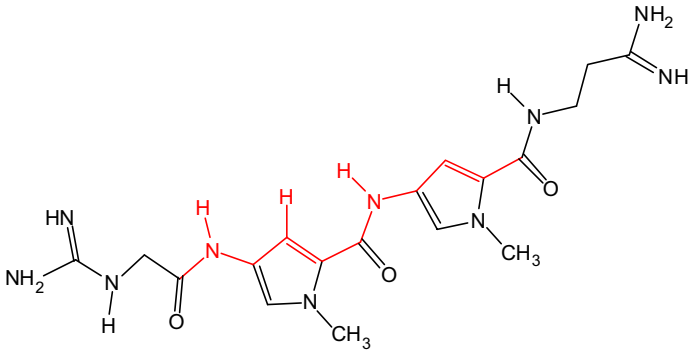
Scheme 1. Synthetic route to benzo[*c*]phenanthridines.

others do not. Compound **7**, the most potent of the analogues against chloroquine-sensitive *P. falciparum*, and the one with the most pronounced cross-resistance, would be prevented from binding in the minor groove by the presence of the furan ring. There is no information available on the mechanism of the observed cross-resistance between chloroquine and the BZPs but the mechanisms of antimalarial action are likely to be very different.

Concerns over toxicity prompted us to assess the activity of five of the new compounds against B82 fibroblast cells in vitro, using a MTT assay. Compounds **7**, **8**, **10** and **11** showed no toxicity up to 100 μg/mL. Compound **9** had LD_{50} 3.2 μg/mL, 2 orders of magnitude higher than the IC_{50} against *P. falciparum*.

Interaction with glutathione is a possibility, given the electrophilic nature of position 6 in the BZPs; chelerythrine has been shown to form adducts with GSH.[14] In our hands, **5** did not react with GSH, even at high pH.

Despite the normal assumption that quaternary salts are excluded from cell contents owing to their low lipid solubility, the original observation of ethnic pharmacology[1] would indicate that BZPs can reach the site of action, in the blood, after oral ingestion. This may be attributable to the equilibrium between the salt and the pseudobase.[8]



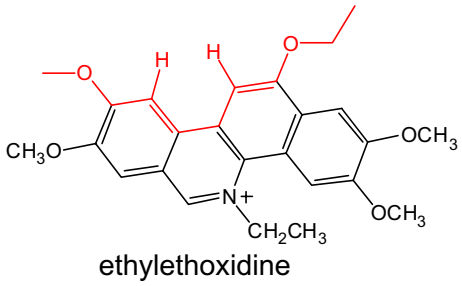


Figure 1. Comparison between the inner faces of netropsin and ethylethoxidine.

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