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Antimalarial effect of bis-pyridinium salts, *N*,*N*′-hexamethylenebis(4-carbamoyl-1-alkylpyridinium bromide)

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Abstract—The in vitro antimalarial activity of bis-pyridinium salts, *N*,*N*′-hexamethylenebis(4-carbamoyl-1-decylpyridinium bromide) and their derivatives, against the *Plasmodium falciparum* FCR-3 strain (ATCC 30932, chloroquine-sensitive) was evaluated. All test compounds exhibited antimalarial activity over a concentration range of 3.5 μM to 10 nM. The chain length of the *N*1-alkyl moiety was found to be very beneficial in terms of antimalarial activity, and in this series of compounds, the most appropriate *N*1-alkyl chain length was found to be eight.

Malaria is one of the most serious parasitic diseases throughout tropical and subtropical regions, and yet it remains a major health problem in developing parts of the world. Every year, 300–500 million people suffer from this disease, and approximately 2 million people die of it.[1] Recently, with the development of various routes of transportation, malaria has become an even larger problem, in that tourists infected with malaria can spread the disease. Furthermore, global warming accelerates this problem. Therefore, malaria is currently an urgent worldwide problem, in addition to being a tropical- and subtropical-specific issue. Furthermore, the spread of malaria strains resistant to chloroquine, mefloquine, and many other available drugs has extremely limited the ability to control this disease. This urgent situation has promoted the necessity for the development of novel antimalarial drugs (Fig. 1).

The histones of *Plasmodium falciparum* have recently been proposed as targets for the treatment of blood-stage parasites.[2,3] Such histones are present in abundance, are antigenic, and may play a role in the pathology of malaria.[3] For example, a naturally occurring compound, apicidin, has been shown to inhibit the in vitro growth of *P. falciparum*.[2] It has been suggested that apicidin acts on parasites by inhibiting histone deacetylase, thus interfering with the continuous acetylation/deacetylation process and preventing cell proliferation. In addition, trichostatin A (TSA), hexamethylenebisacetamide (HMBA), and its analogues such as azelaic bishydroxamic acid (ABHA) and suberohydroxamic acid (SBHA), which are included in inhibitors of histone deacetylase, have also been shown to exert potential activity against *P. falciparum*.[4]

The pharmacological concept of molecule duplication is often an efficient way to identify additional active compounds. *N*,*N*′-Hexamethylenebis(4-carbamoyl-1-decylpyridinium bromide) (**5**) is a recently developed antimicrobial agent.[5] Although this bis-pyridinium salt has a quaternary ammonium moiety, its molecular structure resembles that of HMBA, and this similarity suggests that compound **5** and its derivatives may be useful as antiparasitic agents.

This paper describes the evaluation of the antimalarial activity of compound **5** and its derivatives as novel antimalarial drug candidates against *P. falciparum* in vitro.

Keywords: Antimalaria; Bis-pyridinium salt; *N*,*N*′-Hexamethylenebis(4-carbamoyl-1-alkylpyridinium bromide); *Plasmodium falciparum*; QSAR.
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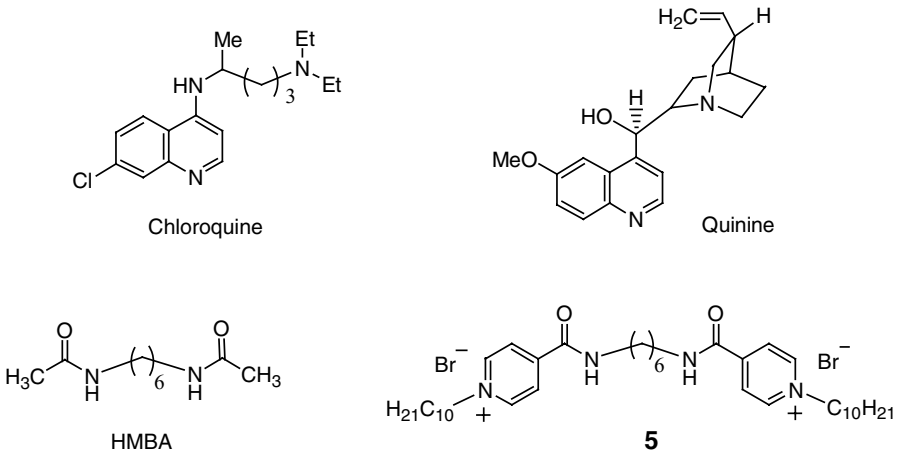
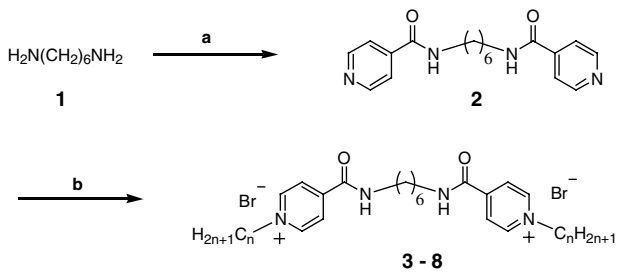


Figure 1. Chemical structures of chloroquine, quinine, HMBA, and compound **5**.

As shown in Scheme 1, bis-pyridinium salts **3**–**8** were prepared according to previously described methods.[5] In brief, isonicotinic acid (**1**) was used as the starting material and was converted into diamide **2** by reflux in sulfonyl chloride followed by reflux with 1,6-diaminohexane in dichloromethane. The resulting diamide **2** was further transformed into the corresponding bis-pyridinium salts **3**–**8** by stirring **2** with 1-bromoalkane in DMF at 100 °C. Elemental analyses, ^{1}H NMR spectra, and other spectral data were consistent in terms of all of the structures examined. The in vitro antimalarial activity of the bis-pyridinium salts **3**–**8** against the *P. falciparum* FCR-3 strain (ATCC 30932, chloroquine-sensitive) and the mammalian cell cytotoxicity of these salts, as determined with the use of mouse mammary tumor cell line FM3A, were assessed according to slightly modified versions of the standard methods.[6] All of the test compounds were assayed in duplicate. Drug-free control cultures were run simultaneously. All data represent means of two experimental assays. The EC_{50} value of the experimental samples was defined by comparison with that of drug-free controls exposed to the same conditions. Selectivity was estimated based on the EC_{50} ratio between malaria parasites and the mouse mammary tumor cell line FM3A, which served as a model host. When the selectivity value exceeded a value of 10, the activity of the compound was considered to be effective. Moreover, the compounds were considered to be very effective when selectivity was >100, according to the present evaluation system.



Scheme 1. Reagents and conditions: (a) 1—isonicotinic acid, $SOCl_2$, in $CHCl_3$, reflux; 2—NaOH, H_2O; (b) $C_nH_{2n+1}Br$, in DMF.

The results are summarized in Table 1. Bis-pyridinium salts **3**–**8**, the chain lengths of which varied with respect to the *N*1-alkyl moiety, exhibited antimalarial activity (EC_{50}) within a concentration range of 3.5 μM to 10 nM, inclusively. Comparison of the EC_{50} values revealed that the highest activity was achieved by compound **4** (EC_{50} = 10 nM), which was approximately 10 times more active than quinine (EC_{50} = 110 nM) and half as active as chloroquine (EC_{50} = 18 nM). On the other hand, the EC_{50} values of these compounds in FM3A cells did not differ substantially. When selective toxicity was used as an index, the highest level of selectivity was recognized in the case of compound **4** (580), which showed about half the selectivity of quinine (910) and one-third that of chloroquine (1800). As regards the issue of *N*1-alkyl chain length, the most potent compound **4** had an octyl group as its *N*1-alkyl moiety. Compound **3**, the *N*1-alkyl chain length of which is shorter than that of compound **4**, exhibited weaker activity than that exhibited by compound **4**. The compounds with *N*1-alkyl chains longer than that of compound **4** were also weaker than compound **4** in terms of activity. In addition, the activity of the compounds weakened progressively with chain lengths longer than 8. Therefore, the chain length of the *N*1-alkyl moiety was found to be crucial with respect to achieving antimalarial activity among the compounds examined here, and the most appropriate *N*1-alkyl chain length was revealed to be 8 (Fig. 2).

Table 1. Antimalarial activity, cytotoxicity, and selectivity of compounds **3**–**8**

Compound	*n*	FCR-3 EC_{50} (nM)	FM3A EC_{50} (nM)	Selectivity
3	6	52	>17,300	>333
4	8	10	5800	580
5	10	40	450	11
6	12	130	420	3
7	14	1100	2800	3
8	16	3500	2200	Non
Chloroquine		18	32,000	1800
Quinine		110	100,000	910

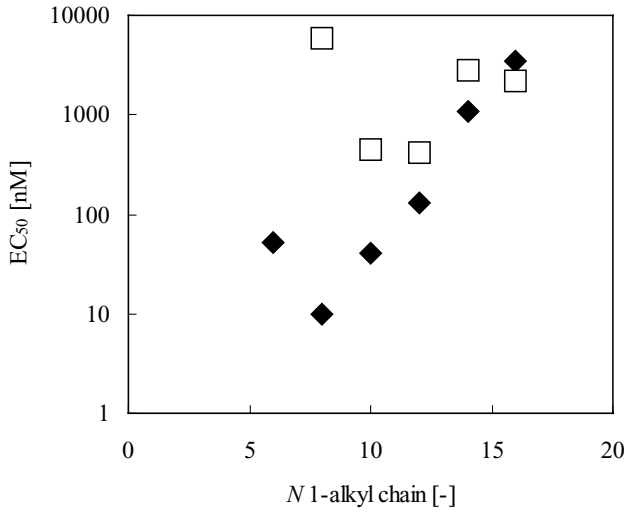


Figure 2. Effect of *N*1-alkyl chain lengths against antimalarial activity (♦) and cytotoxicity (□).

Thus, we prepared a candidate antimalarial agent with potent antimalarial activity and low cytotoxicity, and we observed a structure–activity relationship between the chain length of the *N*1-alkyl moiety and antimalarial activity.

The bis-pyridinium salts investigated in this report are easily prepared in only two steps from isonicotinic acid. Because antimalarial drugs are primarily used in developing countries, easy and inexpensive preparation are important features of potential antimalarial agents. Therefore, based on the present findings, it appears that these bis-pyridinium salts could be greatly advantageous as candidate antimalarial agents.

The study on the mode of action is now in progress.

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